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Effects of Mechanical Stress and Plant Density on Mechanical Characteristics, Growth, and Lifetime Reproduction of Tobacco Plants

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ABSTRACT: Plastic increases in stem elongation in dense vegetation are generally believed to be induced by canopy shading, but because plants protect each other from wind, shielding (reduced mechanical stress) could also play a role. To address this issue, tobacco *Nicotiana tabacum* plants were subjected to two levels of mechanical stress, 0 (control) or 40 (flexed) daily flexures, and grown solitarily, in a dense monostand (with plants of only one mechanical treatment), or in a mixed stand (flexed and control plants grown together). Flexed plants produced shorter and thicker stems with a lower Young's modulus than control plants, while dense-stand plants had relatively taller and thinner stems than solitary ones. Flexing effects on stem characteristics were independent of stand density. Growth, reproduction, and survival of solitary plants were not affected by flexing, while in the monostand growth was slightly reduced. But in the mixed stand, flexed plants were readily shaded by controls and had considerably lower growth, survival, and reproduction rates. These results suggest that wind shielding indeed plays a role in the plastic increase in stem elongation of plants in dense vegetation and that this response can have important consequences for competitive ability and lifetime seed production.

Keywords: allometry, biomechanics, competition, fitness, thigmomorphogenesis, shade avoidance.

In crowded vegetation stands, where light availability declines exponentially from the top toward the bottom of the canopy (Monsi and Saeki 1953), tall plants have a dual advantage in that they are able to position their leaves high in the canopy, where light availability is high, while they can simultaneously shade their shorter neighbors. Consequently, in dense stands taller plants achieve a disproportionate advantage, relative to their size, in terms of light capture (Anten and Hirose 1998), photosynthesis (Hikosaka et al. 1999), and growth and reproduction (Dudley and Schmitt 1996).

Many plant species respond plastically to the proximity of neighbors by producing taller and thinner stems and by a reduction of lateral branches (Weiner and Thomas 1992; Nagashima et al. 1995; Schmitt et al. 1999), a set of responses called the "shade avoidance syndrome." The reduction in the ratio of red to far-red light (R/FR) is generally assumed to be the primary cue that induces these responses (Smith 1982; Schmitt et al. 1999). But other factors, such as blue light perceived by cryptochromes (Ballaré et al. 1991; Ballaré 1999) and neighbor-produced ethylene (Pierik et al. 2003), are also believed to be involved.

Plants cannot violate the laws of physics and must be designed to be able to carry their own weight and resist external wind forces (Niklas 1992). To achieve this, they must invest part of their resources in stems and branches for support and roots for anchorage, and this proportion increases disproportionately with stature (McMahon 1973; Niklas 1992). Taller plants are generally subjected to greater mechanical stress because wind speeds increase with height above the ground surface (Goudriaan 1977) and because the bending moments generated by a given wind force or by the plants' own weight are greater. When plants are exposed to mechanical stimuli, such as wind, touching, or rubbing, they typically produce shorter and

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thicker stems (Biro et al. 1980; Telewski 1990; Jaffe and Forbes 1993; Henry and Thomas 2002) and allocate relatively more mass to roots (Crook and Ennos 1994; Niklas 1998; Henry and Thomas 2002). These responses, collectively known as thigmomorphogenesis (Jaffe 1973; "thigmo" hereafter) increase the resistance of plants to mechanical failure (Niklas 1992) and seem to be in the opposite direction to shade avoidance.

In crowded stands, plants shield each other against wind and wind speeds are usually considerably lower than in open vegetation (Goudriaan 1977; Bertness and Callaway 1994; Speck 2003). Changes in plant allometric growth patterns in dense stands may be elicited not only by canopy shading, blue light, or ethylene but also by a reduction in mechanical stress through wind shielding.

The relative importance of canopy shading and mechanical stress for the changes in the allometric growth patterns has not been investigated extensively (Mitchell 2003). When *Abutilon theophrasti* plants from both open and dense stands were exposed to the same wind treatments, the negative effect of wind exposure on height growth was much stronger for plants from the open stand than for those from the dense stand (Henry and Thomas 2002). A similar interactive effect of stand density and wind on stem allometry was found in three hardwood species (Ashby et al. 1979), suggesting that thigmo is partially suppressed under canopy shading. This could mean that a reduction in height growth in response to wind loading in crowded stands would be selected against because such plants will be outcompeted by others in which thigmo is suppressed. Other studies (Pappas and Mitchell 1985; Mitchell 2003) found that shaded and unshaded plants exhibited similar responses to mechanical stress. Because these studies used neutral shading (shading that did not alter the R/FR ratio), their results are not strong evidence against the hypothesis that shade avoidance in dense stands suppresses thigmo.

We investigated the effects of stand density and mechanical loading on the allometric growth patterns of plants and asked to what extent thigmo is suppressed in dense vegetation. We then estimated the consequences of these responses for both the mechanical stability of whole plants and their lifetime performance in terms of survival and seed production. Quantifying these effects is a crucial step toward understanding the adaptive significance of thigmo and shade avoidance and their possible interaction. We know of only two studies (Niklas 1998; Cipollini 1999) that have attempted to determine the effects of thigmo on reproductive success, and neither studied this in relation to plant density. We exposed tobacco (*Nicotianum tabacum*) plants to two levels of mechanical stress (no stress [control] and 40 stem flexures per day) and grew them under three density treatments: solitary, in dense stands where plants grew together with others that received the same mechanical treatment, and in dense stands where flexed and control plants were mixed.

Material and Methods

Experimental Setup

We conducted two experiments: experiment 1 to determine the extent to which growth responses to mechanical stress (thigmo) are affected by stand density and experiment 2 to quantify the consequences of thigmo for lifetime plant performance (survival, growth, and seed production for both solitary and competing plants). In experiment 1, plants receiving different mechanical treatments were grown separately from each other, while in experiment 2, they were mixed.

Both experiments were carried out in a greenhouse of the Faculty of Biology, Utrecht University, Utrecht, the Netherlands. We used tobacco (*Nicotianum tabacum* cv. Samson N × N), which is an annual plant, so seasonal seed production is a reasonable estimate of fitness. It also has a very simple growth form, bearing its leaves along a single vertical stem, which makes it particularly useful for mechanical analysis. The cv. Samson used in this study generally reaches a height of about 2 m.

Experiment 1: Monostand

On March 9, 2004, seeds were sown in trays in a mixture of sand and potting soil. Seedlings were grown at 30% of natural daylight, a level achieved with neutral-density shade cloth and shading by the greenhouse roof. After 25 days, seedlings were transplanted into pots (3 L) with standard potting soil and without shade cloth, at about 60% of daylight. On April 26, 2004, plant height from soil level to the apical bud, stem diameter just above the first true leaf, leaf number, and the length of the largest leaf were measured for each plant. Plants were ranked from the tallest to the shortest, and the 80 plants of intermediate height were selected for the experiment. An additional group of plants was used as a side row in the high-density treatment. The selected plants were transplanted into pots (3.8 L) made of 40-cm lengths of 11-cm-diameter drainpipe filled with standard potting soil. At this time, we added 4 g of slow-release fertilizer (Osmocote, 10% N + 10% P + 10% K + 3% Mg + trace elements) to each pot (for a total of 0.4 g N plant^{-1}). Plants were watered daily throughout the experiment.

On April 26, 2004, 64 of the 80 plants were randomly assigned to one of two mechanical disturbance (flexing or no flexing) and density (low or high density) treatments (16 plants per treatment). The remaining 16 plants were used for the initial harvest (see below). In the density treatment, plants were grown at either 2 (denoted "solitary" plants) or 81 plants m^{-2} (denoted "monostand" because all plants in a given stand had the same mechanical treatment; see below for the "mixed stand"). The monostand was created by placing four rows of four pots against each other and placing one additional row of plants, not used for any measurement, around the thus-created stands. Solitary plants were far enough apart so that they did not influence each other's light climate. Plants were flexed once a day by gently grasping the stem at about 80% of its height and bending it back and forth no farther than 45° for a total of 40 flexures, which lasted about 90 s. We chose this type of flexing because it simulates the mechanical effect of wind on plants (swaying of the stem) without affecting their microclimate. In the monostand, plants had to be placed apart during flexing, to avoid damaging the other plants. Plants from the nonflexed monostand were also placed away from the other plants for 90 s so that flexing was the only difference in treatment. We randomized the positions of differently treated plants every week within the greenhouse to minimize possible effects of position in the greenhouse.

The first harvest was conducted on April 26, before initiation of treatments, to determine the baseline biomass distribution. The 16 selected plants were cut at ground level and divided into stems and leaves, and fresh weights of both parts were determined. Leaf area was measured with a leaf area meter (LI 3100, LiCor, Lincoln, NE), and the root system was carefully washed. Dry masses of all plant parts were determined after oven drying for at least 72 h at 70°C.

A second harvest was conducted on June 2, 37 days after the initial harvest, to determine growth rates. Plants were cut at ground level, and their height was measured from the base to the top meristem. Basal diameter and the diameters at one-third and two-thirds of stem length were measured to the nearest 0.1 mm with a digital caliper. To determine the vertical distribution of fresh leaf and stem mass, plants were divided into three equal height segments. Leaves of each segment were removed with a razor blade and weighed, and their area was determined as described above. After the mechanical measurements on stems were completed (see below), plants were clipped into the three stem height segments and weighed. Leaf and stem mass of each segment and root mass were determined as described above. Growth rates were calculated by subtracting mass values from the first harvest from those at the final harvest and dividing by the growth period (37 days). For this calculation plants from the first and second harvests were randomly paired.

Experiment 2: Mixed Stand

Experiment 2 was similar to experiment 1. Sowing was done on April 25, 2002, and seedlings were transplanted after 25 days. On June 6, 156 plants of intermediate height were chosen and transplanted into their final pots using the same pots and soil as in experiment 1, but 7 g Osmocote 10 : 10 : 10 (0.7 g N $plant^{-1}$) instead of 4 g was added to each pot because the experiment lasted longer. One day later, 144 of these plants were randomly assigned to the two mechanical stress treatments (0 or 40 daily flexures) and two density treatments (2 or 81 plants m^{-2}), for a total of 36 plants per treatment combination. Twenty-eight of these plants were used to determine growth and reproduction, and eight were used to determine mechanical properties (in experiment 1, measurements of growth and mechanical properties were done on the same plants). The experiment differed from experiment 1 in that flexed and nonflexed plants were mixed in a checkerboard pattern in the high-density treatment. Flexed plants were standing between four nonflexed ones and vice versa. This was done by placing them in two groups of 36 (six rows of six plants) surrounded by side-row plants as in experiment 1. Hence, in the high-density treatment, control and flexed plants were competing for light against each other ("mixed stand"). As in experiment 1, positions of plants were changed once a week to avoid position effects in the greenhouse.

On June 10, before initiation of treatments, 12 plants were harvested to determine the initial biomass distribution, using the same procedure as in experiment 1. Stem height and diameter and leaf and node number were measured on June 21, July 5, and July 24.

A second harvest, 12 plants per treatment combination, was conducted on July 4 to determine growth rates during the first 24 days. The harvest procedure and the method of calculating growth were the same as in experiment 1. A final harvest of 16 plants per treatment combination was conducted on September 20, at the end of the growth cycle, when fruits and seeds had fully matured, to determine lifetime performance of plants in terms of standing biomass and reproduction. In addition to the measurements done during the second harvest, we determined fresh and dry mass of infructescences and fruits. We used total fruit dry mass as a measure of reproductive output.

Mechanical Measurements

Two mechanical properties were measured on both the lower and upper halves of the stems: Young's modulus (E, N m^{-2}) and breaking stress (σ_b, N m^{-2}). Measurements were done in experiment 1 on June 2, 2004, on 16 replicate plants per treatment and in experiment 2 on eight replicate plants per treatment on August 5 and 6, 2002. Leaves were removed from the plants with a razor blade. First the upper half of the stem was cut and used for measurements and then the lower half. All measurements were completed within 15 min after cutting. Stem sections were fixed at both ends between small clamps that were coated with a layer of foam rubber to prevent the tissue from being crushed. To determine E, we placed an empty water bottle exactly halfway along the stem section. Small loads of water (P) were weighed and then added to the bottle, and the vertical displacement (δ) was measured to the nearest millimeter. The advantage of this arrangement is that the force is held perpendicular to the stem even as the stem bends. E could be calculated from the linear regression of δ against P from the equation of a fixed-end beam (eqq. [10]–[19] in Gere and Timoshenko 1999):

$$\delta = \frac{PL^3}{192EI}, \tag{1}$$

where L is the length of the stem segment and I the second moment of area ($I = (\pi/4)R^4$, where R is the stem radius); E is calculated from the slope of this relationship. The break stress (σ_b) was measured by increasing the load in small steps to the point where the stem failed and was calculated as

$$\sigma_b = \frac{MR}{I}, \tag{2a}$$

with

$$M = \frac{PL}{8}, \tag{2b}$$

where M is the bending moment and P, in this case, the load that was supported just before failure occurred (Gere and Timoshenko 1999). There were no significant differences between either the E or the σ_b values of the upper and lower stem sections. Hereafter only the values for the lower stem part are presented and used in the calculations.

Two measures of mechanical stability were calculated with these data: the maximum lateral wind force that plants resist before breaking (F_{max}) and the buckling safety factor. Here F_{max} was calculated as

$$F_{max} = \frac{(1/4)\sigma_b \pi R^3}{H_{lf(av)}}, \tag{3}$$

where $H_{lf(av)}$ is the weighted average height of leaves on the plants. It assumes that wind force acts only on leaves and that all leaves are concentrated at $H_{lf(av)}$, which is calculated as

$$H_{lf(av)} = \sum h_i \frac{A_i}{A_T}, \tag{4}$$

where h_i and A_i are the median height and leaf area of segment i (see above for harvesting methods) and A_T is the total leaf area of the entire plant. Note that equation (3) treats wind loading as a static phenomenon, ignoring its dynamic nature (Baker 1995).

The buckling safety factor (BSF) was calculated as the critical buckling height (H_c) of the plant divided by its real height (H_{real}):

$$\text{BSF} = \frac{H_c}{H_{real}}, \tag{5a}$$

where H_c was calculated using the formula of Greenhill (1881) for a uniform column,

$$H_c = 1.26\left(\frac{E}{\rho}\right)^{1/3} d^{2/3}, \tag{5b}$$

with ρ the fresh weight per unit stem volume (N m^{-3}). This formula treats stems as idealized columns, ignoring tapering and uneven loading. However, its results tend to be comparable to those of more complicated models (Holbrook and Putz 1989; Henry and Thomas 2002). In general, the use of simplified mechanical models such as equations (3) and (5) is sufficient for the qualitative comparison of mechanical stability between plants of the same species with a very similar basic structure, as was done here.

Statistical Analysis

A two-way ANOVA was used to test for differences in response parameters, with mechanical treatment (df = 1) and stand density (df = 1) as fixed factors. Data transformation was based first on Levene's test for equality of variance and second on the Shapiro-Wilk test of normality.

Results

Experiment 1

Flexed plants produced stems that were 40% shorter, 7% thicker, and therefore considerably less slender (height/diameter) than control plants. Stand density had an opposite effect; plants from the monostand had taller and thinner stems than solitary plants (fig. 1; table A1 in the online edition of the *American Naturalist*). There was no significant mechanical stress × density interactive effect on either height or diameter; flexing had very similar ef-

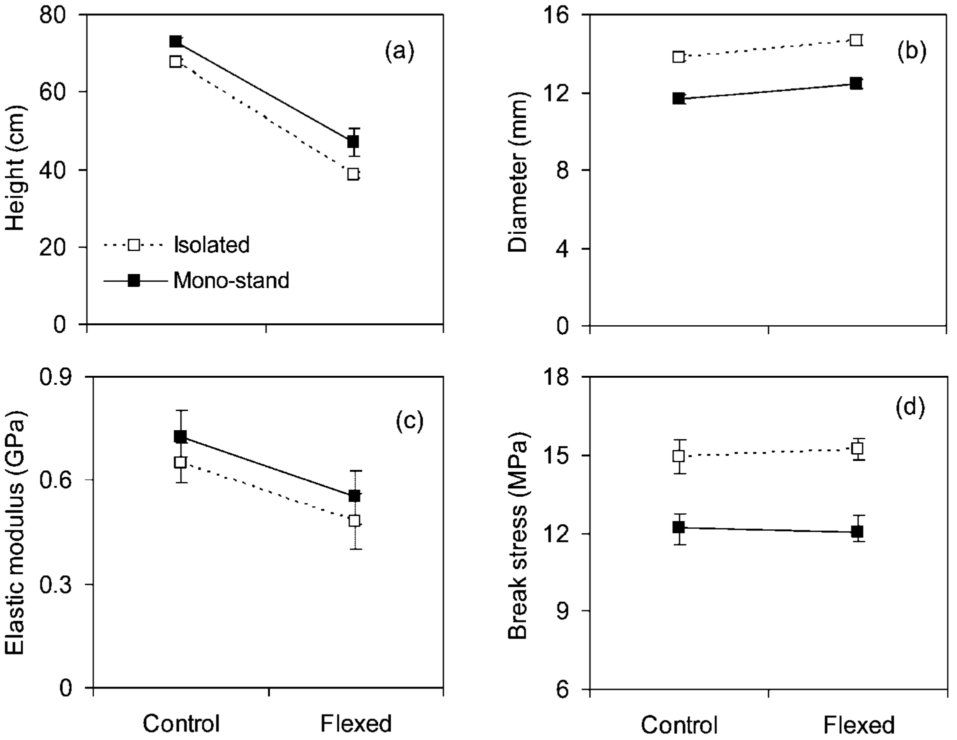

Figure 1: Height (*a*), diameter (*b*), Young's modulus (*c*), and break stress (*d*) of flexed and nonflexed (control) tobacco plants grown at two densities: 2 (solitary, plants do not shade one another) and 81 (monostand) plants m^{-2}. All data are taken from experiment 1. Monostand implies that flexed and control plants were not mixed. Bars indicate standard errors ($n = 16$).

fects on these two parameters under both densities (table A1). This suggests that thigmomorphogenic changes in stem height : diameter allometry were not suppressed under canopy shading in the monostand.

The Young's modulus of stems (E) was lower in the flexed than in the control plants but was not affected by stand density (fig. 1; table A2 in the online edition of the *American Naturalist*). The breaking stress (σ_b), on the other hand, was lower for the monostand plants than for the solitary ones but did not differ between flexed and control plants (fig. 1; table A2).

Flexed plants had 75%–100% greater maximum lateral force values (F_{max}; eq. [3]) than control plants and significantly larger buckling safety factors (BSFs; eq. [5]; fig. 2; table A2). These differences were the result of the shorter stature and greater stem diameter of flexed plants. Plants from the monostand were calculated to have lower F_{max} and BSF values than solitary plants.

Solitary flexed plants grew at the same rate as controls. Among the plants in the monostand, however, growth of flexed plants was about 20% slower (fig. 3; table A2). Flexed plants allocated larger fractions of their mass to roots and leaves and less to stems (fig. 4; table A2).

Experiment 2

As in experiment 1, flexed plants were generally shorter and had larger stem diameters than control plants, although the effect on height was less pronounced than in experiment 1. Among the solitary plants, the difference in height between flexed and control plants increased to about 16 cm on July 5 and then remained constant until the end of the experiment (data not shown). Because of their slower height growth, flexed plants in the mixed stand readily became shaded by the control plants, and stem characteristics in these plants were partly the result of shading. This made it difficult to interpret density × mechanical stress interactions on stem traits. The difference in diameter was only significant on June 21 and July 24, not on the other two dates (data not shown).

As in experiment 1, flexed plants produced stems with a lower Young's modulus (E) than control plants (table 1). Among the solitary plants, σ_b was not significantly affected by flexing. There were no significant differences among the control plants in either E or σ_b between the plants grown at different densities ($P > .05$, Studentized t-test).

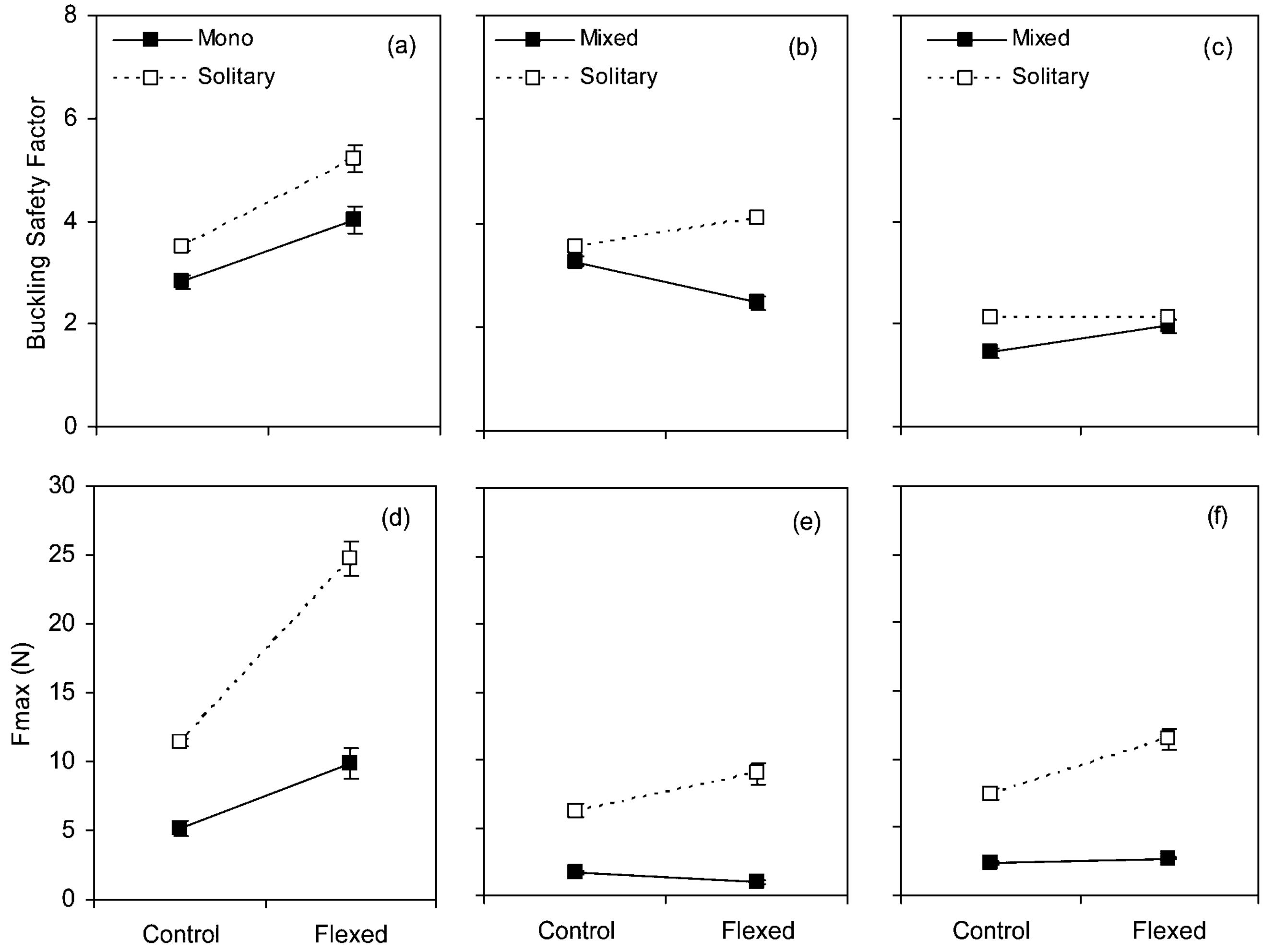

Figure 2: Buckling safety factors (*a*–*c*) and maximum lateral force that plants can resist (F_{max}; *d*–*f*) calculated for tobacco plants from experiment 1 (*a*, *d*) and from the July 4 (*b*, *e*) and September 20 (*c*, *f*) harvests in experiment 2. Plants were either flexed or not flexed (control) and grown at two densities: 2 (solitary, both experiments) and 81 plants m^{-2} (monostand in experiment 1: flexed and control plants grown separately; mixed stand in experiment 2: flexed and control plants grown together). Bars indicate standard errors ($n = 16$ in *a*, *c*, *d*, and *f* and 12 in *b* and *e*).

The buckling safety factor (BSF) and maximum lateral force that plants can resist (F_{max}) were calculated for both the second harvest, 24 days after treatment initiation, and the final harvest. Among the solitary plants at both harvests, flexed plants were calculated to have about 40% higher F_{max} values than control plants (fig. 2; $P < .05$, Studentized *t*-test). The BSF values of flexed solitary plants were higher than those of controls in the second but not the final harvest. Among the mixed-stand plants, however, F_{max} values of flexed plants were lower than those of controls at the first harvest and not different at the final harvest (fig. 2).

Growth rates of solitary plants were not affected by flexing during the first 24 days after initiation of treatments. Within the mixed stand, on the other hand, flexed plants grew at a rate barely one-sixth that of control plants (fig. 3; table A2). Contrary to the results in experiment 1, flexing had no effect on the fractional allocation of mass to roots. Fractional allocation to leaves, however, increased, and allocation to stems decreased significantly (table A2).

For the solitary plants, flexing did not have an effect on lifetime seed production and survival probabilities (fig. 5); all plants survived, and total reproductive biomass, the fraction of total biomass in reproductive organs (ReMR), and final standing mass were not significantly different between the two treatments ($P = .687$, Studentized *t*-test). In addition, there were no differences in the average number of days until flowering (84.5 ± 0.5 and 85.2 ± 0.7, for the control and flexed plants, respectively). In the

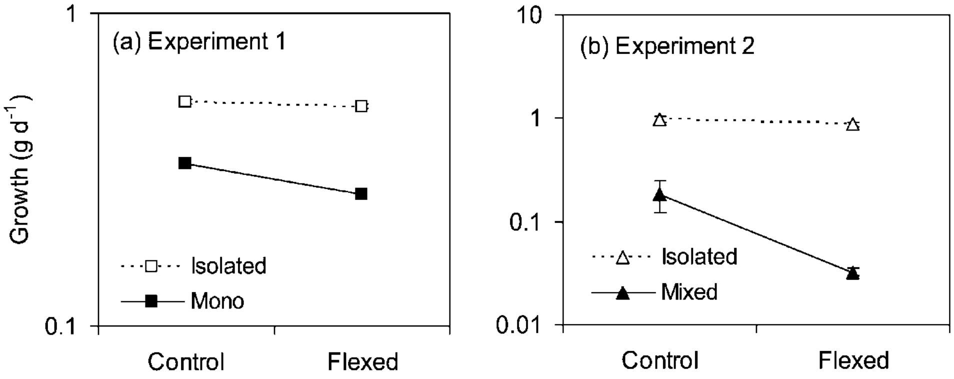

Figure 3: Growth rates of tobacco plants from experiments 1 (*a*) and 2 (*b*) during the first 24 days after treatment initiation. For description of treatments, see figure 2 and main text. Bars indicate standard errors ($n = 16$ in *a* and 12 in *b*).

mixed stand, on the other hand, lifetime performance was much lower for the flexed plants than for the controls. Only 19% of the flexed plants survived, as compared to 92% of controls, while none of the flexed plants produced seeds (control plants produced about 6 g of seeds; fig. 5).

Discussion

Interactive Effects of Flexing and Canopy Shading on Growth Characteristics

Mechanically stressed plants produced shorter, thicker stems made of less rigid tissue (lower Young's modulus, *E*) and allocated more mass to roots than control plants. Similar responses have been observed in other studies (e.g., Jaffe and Forbes 1993; Niklas 1998; Mitchell 2003), and they most likely increase the mechanical stability of plants (Niklas 1992, 1998).

Among the solitary plants in experiment 2, the difference in height between flexed and control plants increased to about 16 cm during the first 28 days of the experiment, after which it remained constant. Apparently, plants responded to mechanical stimulation with a reduction in the rate of stem elongation only up to a certain age but not beyond that. This is consistent with the notion that plasticity can vary across ontogeny (Bradshaw 1965). It has, for example, been found that plasticity in stem elongation in response to light quality decreases during development (Causin and Wulff 2003) and that this might be adaptive in certain habitats (Weinig 2000).

As a result of their lower stature and greater stem girth, flexed plants should be able to resist considerably greater lateral wind forces ($F_{\max}$) and be better able to carry their own weight, as reflected in their higher calculated buckling safety factors (BSFs; fig. 2). The lower *E* values contributed negatively to the flexed plants' ability to carry their own weight (see eq. [5]) but could be considered an adaptation to windy conditions. This is because more flexible stems bend more easily in the wind and hence convert a smaller bending moment to their bases (Niklas 1998). We did not consider this effect in our calculation of $F_{\max}$, as we assumed that plants rigidly maintain their vertical posture under wind loading. The relatively larger root system was also not included in our calculations, but it may provide greater stability because greater force would be required to uproot the plant (Goodman and Ennos 1996). Overall, this indicates that the difference in wind resistance ($F_{\max}$) between flexed and control plants was greater than we estimated.

Changes in Young's modulus (*E*) were probably associated with changes in the stem anatomy. For example, lower *E* values of stems of flexed *Capsella bursa-pastoris* plants were associated with a relative increase in parenchyma cells and a reduction in phloem fibers (K. J. Niklas, personal communication), with the former having a much lower *E* than the latter (Niklas 1993).

In this study, manual flexing was applied to simulate the mechanical stress caused by wind loading. But air flow affects plant growth in other ways, too, for example, through changes in the microclimate of leaves or by folding leaves, hence reducing their photosynthesis (Ennos 1997). A recent study (Smith and Ennos 2003) showed that air flow per se induced increased stem elongation, an effect opposite that of mechanical stress. A combination of flexing and airflow, however, caused a reduction in stem elongation, suggesting that the mechanical effect of wind is stronger than its physiological effect (Smith and Ennos 2003). This is consistent with the fact that in most studies, plants exposed to the full effect of wind are found to be shorter than wind-shielded individuals (Holbrook and Putz 1989; Henry and Thomas 2002).

The responses of plants to mechanical stress were in-

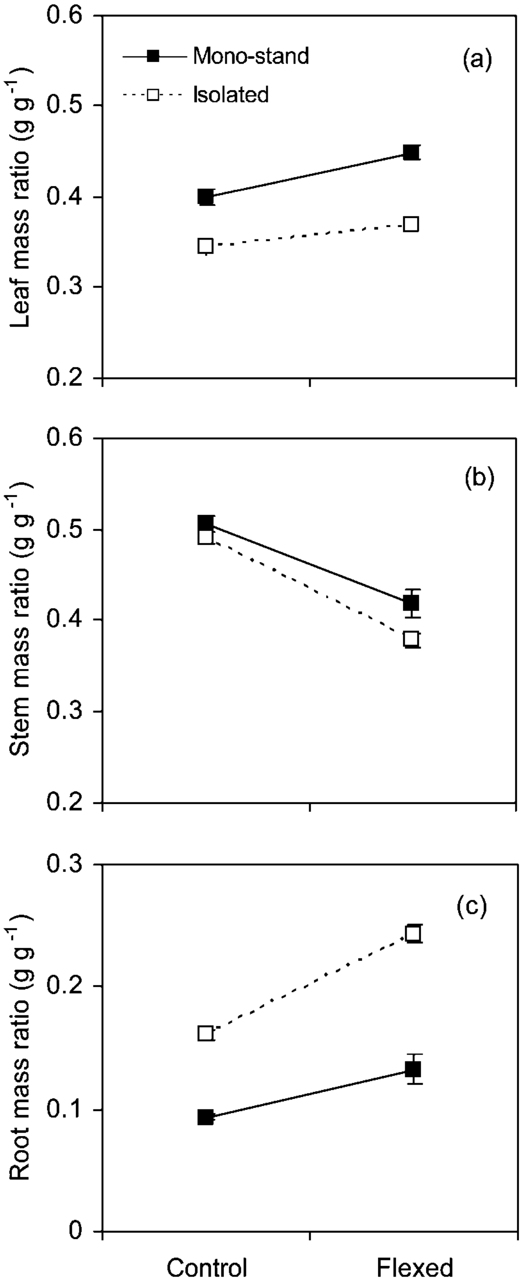

Figure 4: Leaf (*a*), stem (*b*), and root (*c*) mass ratios of tobacco plants from experiment 1. Description of treatments is given in figure 1 and in the main text. Bars indicate standard errors ($n = 16$).

dependent of stand density. Increased density had an effect on plants that was opposite in nature to that of mechanical stress, with high-density plants producing taller, thinner stems and relatively fewer roots than solitary individuals. This response, generally denoted as shade avoidance, has been observed in many other studies (see the introduction to this article). But the thigmomorphogenic responses did not differ between the density treatments; in both density treatments in experiment 1, we observed similar reductions in stem length and Young's moduli and increases in stem girth and root allocation. These results are contrary to those of Ashby et al. (1979) and Henry and Thomas (2002), who suggested that thigmo is suppressed when plants grow in dense stands, pointing out that this would be an adaptive response because shorter stature would confer a large disadvantage under these conditions.

It has been noted that the effects of density on the allometry of plant stems can be confounded with the effects of mechanical stress (Holbrook and Putz 1989; Mitchell 2003). In dense vegetation stands, plants shield each other against wind, and wind speeds can be considerably lower than in an open habitat (see the introduction to this article). Wind speeds within a dense tobacco stand grown outdoors were measured to be one-fourth to one-fifth those outside the stand (N. P. R. Anten, unpublished data). Thus, plants within such stands are probably subjected to similarly lower lateral forces and associated flexing, since wind force scales more or less linearly with wind speed (Vogel 1994; for tobacco, N. P. R. Anten, unpublished data). Our experiment was conducted within a greenhouse, a wind-protected environment, and the control plants can therefore be interpreted as wind-shielded plants. In the dense stands created in the experiment, the control plants experienced both canopy shading and wind shielding, while the flexed plants experienced canopy shielding but were exposed to a type of mechanical stress, flexing, that was similar to the stress associated with wind loading. Consequently, the differences between flexed and control plants observed in the dense stands suggest that plants in crowded stands produce taller and thinner stems, partly in response to changes in light quality and neighbor-produced ethylene mentioned in the first section of this article and partly in response to wind shielding.

A further argument for why one would expect plants in crowded stands to maintain their sensitivity to mechanical stress comes from the so-called height convergence of upper plants observed in such stands. In dense stands of herbaceous plants, the tallest individuals are generally of very similar height, while they may differ considerably in terms of stem diameter, leaf area, and plant mass (Weiner and Thomas 1992; Nagashima and Terashima 1995; Nagashima et al. 1995). What prevents the more massive individuals, with more leaf area and greater growth potential, from growing out above the rest of the vegetation? Wind speeds increase drastically with increasing height above the top of the vegetation (Goudriaan 1977; Bertness and Callaway 1994). A twofold increase in wind speeds within the first 0.4 m above the canopy of a 3-m-tall *Arundo donax* has been recorded (Speck 2003).

Table 1: Stem characteristics measured on control and flexed tobacco plants grown either in isolation or in a mixed stand (experiment 2)

	Isolation		Mixed Stand	
	Control	Flexed	Control	Flexed
Mechanical properties:				
Height (cm)	84.3 (3.8)	67.3 (2.8)	60.5 (4.6)	27.0 (1.1)
Diameter (mm)	13.3 (.33)	14.8 (.51)	8.4 (.41)	7.1 (.27)
σ_b (MPa)	10.7 (.5)	9.3 (.5)	10.1 (3.6)	4.3 (2.5)
E (GPa)	1.55 (.15)	.93 (.07)	1.11 (.39)	.09 (.05)
Growth data:				
Leaf mass ratio	.469 (.013)	.489 (.012)	.491 (.016)	.544 (.008)
Stem mass ratio	.379 (.011)	.349 (.015)	.378 (.008)	.344 (.009)
Root mass ratio	.151 (.018)	.164 (.024)	.129 (.017)	.111 (.011)

Note: In the mixed stand, flexed and control plants were mixed together. Values in parentheses denote SEMs ($n = 12$ except for σ_b and E, for which $n = 8$). The properties σ_b and E are the breaking stress and Young's modulus of the stem, respectively.

Similarly, wind speeds more than doubled within 0.3 m above a field-grown 1.8-m-tall tobacco stand (N. P. R. Anten, unpublished data). It is possible that the increased mechanical stress and flexing that plants experience when they grow out above the vegetation line induces a reduction in height growth and an increase in diameter increment and root allocation. Plants that grow out above the vegetation, however, may also be exposed to light with a higher red–far-red ratio, which could also inhibit height growth (Ballaré 1999). The degree to which this occurs depends on the spatial distribution of the phytochromes and their regulation along the plant (Reddy and Sharma 1998).

Consequences for Lifetime Performance

Fitness costs associated with thigmo could not be detected for solitary plants, among which flexed plants grew and produced reproductive biomass at a similar rate, flowered at the same date, and had the same survival probabilities as control plants. This result contradicts the few other studies (Niklas 1998; Cipollini 1999) that have estimated the consequences of thigmo for reproductive success, which found that mechanically stressed plants produced fewer flowers and flowered at a later date than untouched individuals. Cipollini (1999) pointed out that the reduction in flower production was the result of internal resource allocation trade-offs; resources allocated to increased mechanical stability could not simultaneously be allocated to leaves or reproduction. In our study, flexed plants tended to allocate more mass to roots but less to stems than control plants, but neither the fraction of mass allocated to leaf production nor that allocated to reproduction was negatively affected (table 1; fig. 4), suggesting that there was no such trade-off. Cipollini (1999), however, argued that thigmo has been shown to involve the production of a number of strength-enhancing proteins (Braam et al. 1996) that may be comparatively costly to produce and that this cost might be reflected in a lower seed production. It would indeed be interesting to compare the construction costs and maintenance respiration of support structures between flexed and unflexed plants. Yet even if such a cost exists, it apparently did not hamper seed production in our study.

Contrary to the results for the solitary plants, flexed plants in the mixed stand in experiment 2 (where flexed and control were grown together) had dramatically lower growth, reproduction, and survival rates than the undisturbed ones. This result cannot be attributed to a direct effect of mechanical stress on growth but was a direct consequence of shading. Height growth of control plants was 25% greater than that of flexed plants. Consequently, flexed plants were progressively shaded by their untouched neighbors, and this strongly reduced their vigor to grow and reproduce. These results clearly confirm the notion derived from previous studies that in crowded stands, small differences in height growth have profound fitness consequences (Dudley and Schmitt 1996; Schmitt et al. 1999; Pierik et al. 2003).

In open habitats, plants are likely to be exposed to relatively large wind forces. Our results show that in response they can increase their ability to resist wind, as expressed by the calculated maximum lateral force that plants can resist (F_{max}), apparently at little or no fitness costs. By contrast, in dense vegetation, plants are relatively protected against wind. Our data indicate that they are able to respond plastically to this lack of mechanical stress and associated flexing by increasing their rate of height increment. This cue acts in addition to other cues that may induce stem elongation. The large difference in lifetime performance between flexed and unflexed plants in the

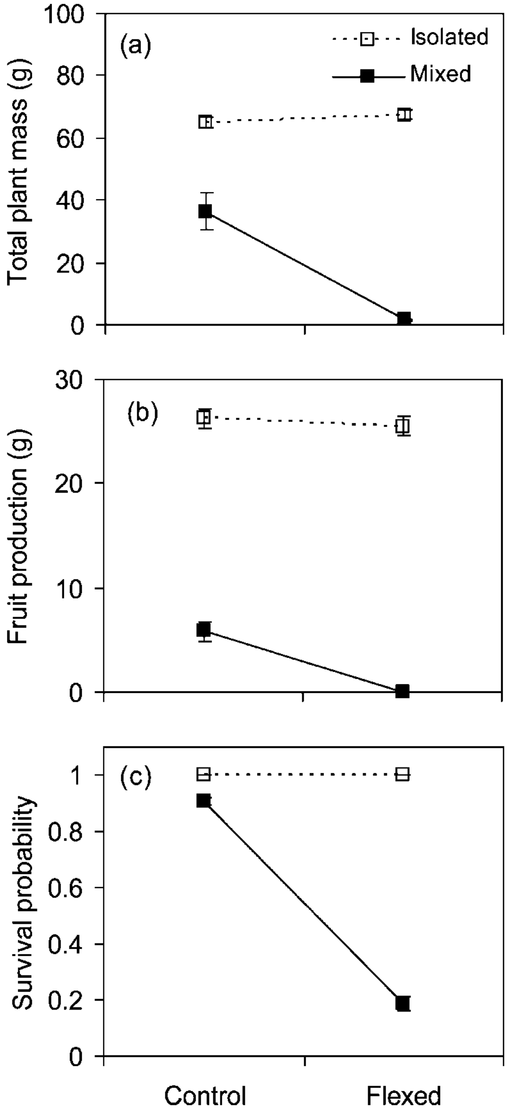

Figure 5: Final standing biomass (*a*), lifetime fruit production (*b*), and survival rate (*c*) of flexed and nonflexed (control) tobacco plants grown at two densities: 2 (solitary, plants do not shade one another) and 81 (mixed stand) plants m^{-2}. All data are taken from experiment 2. Mixed stand implies that flexed and control plants were mixed. Bars indicate standard errors ($n = 16$).

mixed stand further indicates that there is a large fitness premium on this response to wind shielding.

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