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Snap, crack and pop of explosive fruit

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There is an increasing appreciation for the role of physical forces in plant development. Mechanics are fundamental to how explosive fruit eject their seeds, and recent studies have successfully combined mechanics with developmental genetics to help explain how these dispersal traits are produced and how they evolved. Computational modeling is used more and more to address developmental questions, and explosive fruit are particularly good systems for combining biology and modeling approaches. Finite element models have been recently used to explore questions such as: Why do touch-me-not species with similar fruits, differ so much in how efficiently they transfer stored energy to eject seeds? And how do popping cress fruits use the expansive force of turgor pressure for tissue contraction?

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Introduction

It is tough for a small seed to make it in the big world! Not only does it need suitable conditions to grow, but it also faces competition from other plants for these resources. Parent and offspring are usually at a disadvantage if they compete for resources. This is one reason why all organisms show a tendency to disperse away from their parents [1]. Evolution has shaped a number of ingenious adaptations for plants to disperse their seeds and fruits [2,3]. For example, seeds that autorotate or glide have a long descent time so that the wind can disperse them away from their tree of origin. Autorotating seeds of the maple tree (*Acer pseudo-platanus*) attain high lift by generating a stable leading-edge vortex, similar to hovering insects [4], while the gliding flight of large Javan cucumber seeds (*Alsomitra macrocarpa*) was the inspiration for early aviation pioneers [5]. Other plants produce fruit to encourage animals to eat and then disperse their seeds to new locations; for example, the African savanna elephant can disperse seeds over long distances up to 65 km [6]. Still other fruits and seeds attach to their animal dispersers, typically by hooks or spines [7]; for example, burdock burrs (*Arctium* spp.) were the inspiration for Velcro design [8]. But perhaps most extraordinary, are the fruits that generate their own explosive force to fling their seeds away.

Seeds are often very small and immediately slowed down by aerodynamic drag. To get such small particles airborne requires rapid acceleration. Unlike animals, plants do not have muscles, and need to rely on entirely different mechanisms than motor proteins to move their organs. These mechanisms typically exploit the forces generated by water movement and involve differential growth, or reversible swelling/shrinking of cells (turgor-driven movements) or cell walls (humidity-regulated movements) [9]. However, these processes are constrained by the maximum speed of water transport across cells and tissues, and are not rapid enough to explain explosive movements at a millisecond timescale [10,11]. Plants can employ elastic instabilities to generate very rapid motions. These rely on a geometry capable of gradually storing elastic energy and then rapidly releasing it. Since the motion itself does not involve water movement, its speed is now constrained by the speed of elastic waves in the tissue material [10]. Two main types of elastic instabilities used by plants are snap-buckling and explosive fracture. Snap-buckling involves the rapid geometric change of a thin shell that does not rupture, and explains the snap of the bladderwort suction cup [12] and the Venus fly trap [13]. Explosive fracture involves a rapid geometric change that cracks the plant tissue, and is found in many explosive fruit such as the touch-me-not plant *Impatiens glandulifera* [14].

Previous studies of explosive fruit have focused on characterizing the mechanics of explosive opening and identifying the elastic instabilities associated with these movements (reviewed in [15,16•,17]). Because these movements are so fast, it was a challenge for inventive scientists during the past century to measure and record explosive seed dispersal before these events could be accurately filmed with high-speed cameras [18–24]. More recent studies have taken a multi-disciplinary approach to gain a comprehensive understanding of explosive fruit opening [25••]. This task is non-trivial. A key problem is that model species like *Arabidopsis thaliana*, where the experimental tools for detailed functional studies exist, do not exhibit rapid movements such as explosive seed dispersal. A fundamental theoretical challenge is that rapid movements are the culmination of activities integrated across different spatial scales, hence a complete understanding requires biomechanical models that link causal events at the cell and tissue levels to the macroscopic organ and plant response. To address these issues, scientists recently combined theoretical models at different spatial scales with genetic studies in *Cardamine hirsuta*, a close relative of *A. thaliana* that uses explosive fruit to eject its seeds [25••,26,27]. In this review, we will discuss work over the past two years that has focused on the mechanics of explosive seed dispersal in various fruit.

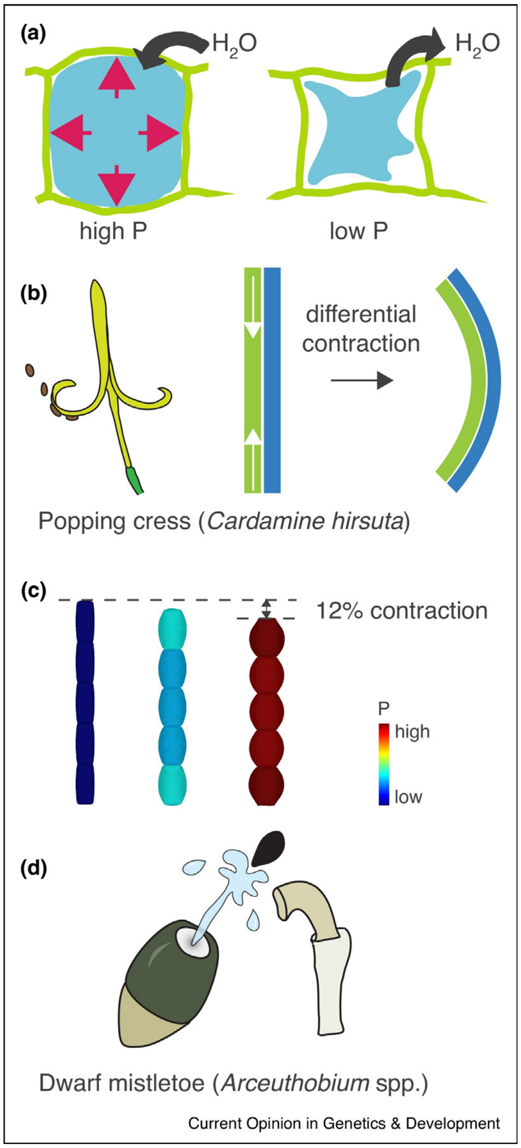
Pump up the volume

While cells can be described as 'the little bags of water you're made of' [28], plant cells are better described as little pressure bombs [29]. Fully hydrated plant cells are under high internal pressure, commonly 0.4–0.8 MPa, but can reach up to 4 MPa in the guard cells of stomatal pores [11]. Plant cells are prevented from bursting under this pressure by a strong, rigid cell wall. This hydrostatic pressure, called turgor pressure, pushes the plasma membrane against the cell wall (red arrows, Figure 1a). To balance this pressure, in-plane mechanical stresses develop in the cell wall. Changes in turgor pressure occur by the exchange of water between a cell and its environment due to evaporation or osmosis (Figure 1a). In this way, water flows towards compartments where the water potential is lowest [29]. These physical properties — water flow induced by gradients of water potential, turgor pressure, and cell-wall deformation — form the basis of water-driven movements in plants.

Explosive fruit rely on hydraulics to gradually store elastic energy that is suddenly released once certain design features undergo mechanical failure. Typically, dehydration causes fruit tissues to deform, creating tension as cells contract when they lose turgor pressure through passive drying (Figure 1a). Passive rather than active contraction is typical for explosive seed dispersal since these are irreversible movements that tear the fruit tissue [30]. However, Hofhuis *et al.* found that this concept does not apply to the explosive fruit of popping cress (*C. hirsuta*). For one thing, *C. hirsuta* fruit explode while the tissues are turgid, not dry [18], and moreover, differential contraction of the fruit wall requires living cells that sustain high turgor pressure [25••].

During explosive seed dispersal, the fruit walls of the *C. hirsuta* fruit pod coil rapidly, transferring kinetic energy to the seeds to fire them away (Figure 1b). Coiling is a consequence of the bi-layer configuration of the fruit wall (Figure 1b). An active, outer tissue layer contracts (green, Figure 1b) while an inextensible inner layer, stiffened by lignin, does not (blue, Figure 1b). This differential contraction causes the initially straight valve to coil (Figure 1b). But how do turgid cells contract in length? Hofhuis *et al.* used quantitative imaging and osmotic treatments to show that the exocarp cells of *C. hirsuta* fruit respond anisotropically to turgor pressure, contracting in length, while expanding in depth and width [25••].

Figure 1



Turgor pressure drives active contraction in the *C. hirsuta* fruit wall. **(a)** Plant cell under high turgor pressure (high P, left) due to water influx. The cell expands, pushing the plasma membrane against the cell wall (red arrows). Water efflux results in low turgor pressure (low P, right), causing the plasma membrane to shrink in from the cell wall and the cell to contract. **(b)** Explosive coiling of the fruit walls ejects the seeds from a *C. hirsuta* fruit pod (left). Each fruit wall is arranged as a bilayer with an outer layer (green) that contracts (white arrows) and an inner layer (blue) that does not. This differential contraction causes the initially straight fruit wall to coil (right). **(c)** Finite element model simulations of *C. hirsuta* fruit exocarp cells pressurized from low (blue) to high (red) turgor pressure (P) (data reproduced from [25••]). Dashed lines mark the initial length of the cell file and the final length; arrows indicate 12% contraction in length. **(d)** Osmotic pressure drives the explosion of dwarf mistletoe (*Arceuthobium americanum*) fruit, which ejects the seeds with a stream of mucilage.

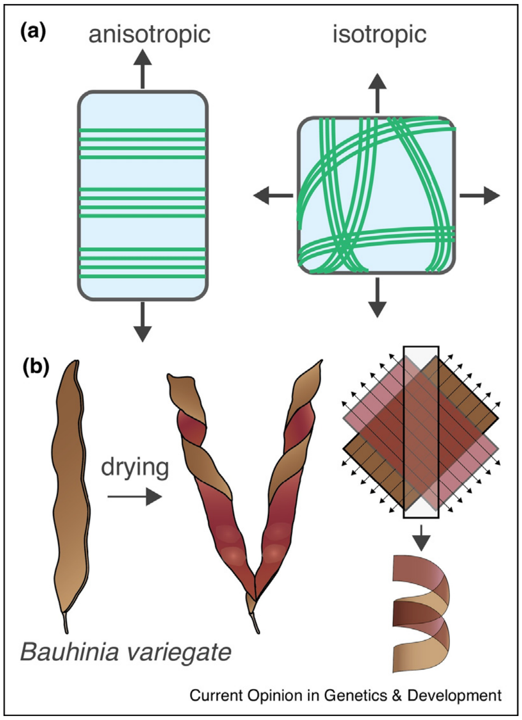
They could reproduce this response in a finite element model that used anisotropic cell wall material and the actual 3-dimensional geometry of *C. hirsuta* exocarp cells (Figure 1c) ([25••] and associated video abstract). This specific cell shape is conserved between *C. hirsuta* and other *Cardamine* species with explosive fruit [31•], suggesting that they may share a common mechanism of active contraction to power explosion. Future work could investigate the generality of this mechanism in *C. parviflora* [24] and other fruit that employ explosive fracture such as *I. glandulifera* [14] and *I. capensis* [23].

Hydrostatic pressure is used to even greater effect in other explosive fruit. A very high osmotic pressure builds up in the fruit of squirting cucumber (*Ecballium elaterium*) and dwarf mistletoes (*Arceuthobium* spp.), which ultimately explode, dispersing seeds with a stream of mucilage (Figure 1d). This mechanism for rapid seed ejection has recently inspired thermo-triggered squirting capsules for nanoparticle delivery with potential applications in biomedicine [32]. In dwarf mistletoes, a mucilaginous layer of viscin cells, between the seed and the fruit exocarp, accumulates considerable osmotic pressure until finally, the fruit fractures along its abscission zone. The exocarp then contracts rapidly, hurling the seed upward with initial velocities estimated close to 50 km/h [19] dispersing seeds almost 15 m away [20]. More recently, deBruyn *et al.* measured endogenous heat release in dwarf mistletoe (*Arceuthobium americanum*) fruit, and an associated increase in fruit surface temperature, just minutes before explosive discharge [33•]. Interestingly, these results may mean that thermogenesis can trigger the explosive fracture of the fruit along its abscission zone [33•]. It remains to be determined whether any link exists between endogenous heat production and ambient temperature, but enhanced seed dispersal of this parasitic plant under global warming would have considerable implications for the forestry industry.

Do the twist

Plant movements depend on both turgor pressure and cell wall mechanics. A plant cell wall can be viewed as a fibre-reinforced gel; composed of rigid cellulose microfibrils embedded in a hydrated matrix of polysaccharides [34]. The orientation of cellulose fibres in the cell wall influences the direction of expansion in living cells (Figure 2a), but also directs movements that occur in dead tissues. The cells walls of fibrous plant tissues are hygroscopic and can swell or shrink depending on humidity [9]. According to the arrangement of cellulose fibres, this can translate into organ movements, such as the opening of pine cone scales [35], and the explosive dispersal and self-burial of awned seeds of *Erodium cicutarium* [36] and *Pelargonium peltatum* [37]. The fruit pod of the orchid tree (*Bauhinia variegate*), for example, opens by a chirality-creating mechanism that turns an initially flat fruit valve into a helix (left, Figure 2b) [38]. The fruit valves consist of two fibrous layers, with cellulose microfibrils oriented approximately 45° with respect to the pod's longitudinal axis. Upon drying, these two layers shrink in perpendicular directions, which is sufficient to drive the flat-to-helical transition that opens the fruit pod. As proof of concept, the authors mimicked this structure with a mechanical analog comprising two pre-stretched latex sheets that shrunk uniaxially in perpendicular directions [38]. A strip cut from this material curled into a helical configuration (right, Figure 2b), reproducing the geometry of the *B. variegate* fruit pod.

Figure 2

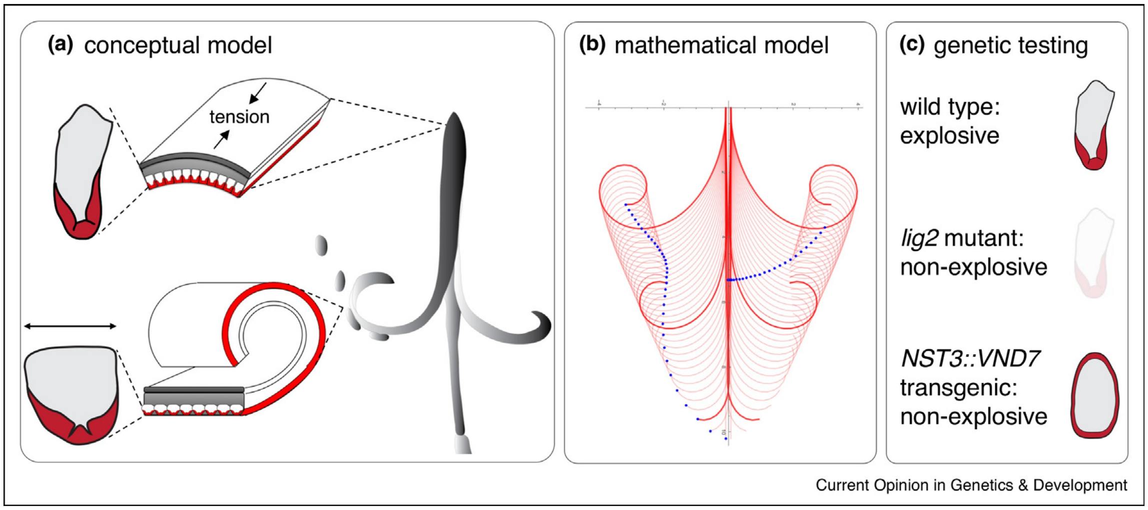


The orientation of cellulose microfibrils in the cell wall influence directional growth and hygroscopic movements. **(a)** Cellulose microfibrils (green) are aligned perpendicular to the direction of growth in anisotropically growing cells (left) but not in isotropically growing cells (right). **(b)** Fruit pod opening in *Bauhinia variegate* involves a hygroscopic movement that turns a flat valve into a helix (left). Each fruit valve has two layers (shown as brown and red) with cellulose microfibrils oriented roughly 45° with respect to the pod's longitudinal axis. Upon drying, these two layers shrink in perpendicular directions, which is sufficient to drive the flat-to-helical transition. This structure was mimicked using composite elastic materials (right) where two layers (brown and red) were pre-stretched (directions indicated by arrows) and glued together such that the layers shrunk uniaxially in perpendicular directions. A strip cut from this material (indicated by box) curls into a helical configuration [38].

A need for speed

Rapid plant movements depend on the fast release of stored elastic energy; and mechanisms that employ explosive fracture are the fastest of these movements [10]. Two recent studies have highlighted the sophisticated design

Figure 3



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Multi-scale model reproduces explosive seed dispersal in *C. hirsuta*. **(a)** At the organ scale, tension is generated by differential contraction of valve tissues. Explosive release of this tension is controlled at the cellular scale by asymmetric lignification (red) of endocarp *b*cells in the valve. These lignified cell walls are shaped like a hinge, which can open (arrow). The valve needs to coil along its length to reach its relaxed state, but its transverse curvature prevents this. Opening the hinge flattens the cross-section of the valve, which removes this energy barrier, allowing the sudden release of stored elastic energy. **(b)** Formalizing these concepts in a mathematical model of the elastic energy in the fruit valves describes the dynamics of the coiling valves. Figure reproduced from Ref. [25••]. **(c)** Key predictions of the mathematical model were tested using genetics. Compared to the hinged secondary cell wall in endocarp *b* cells of explosive wild-type fruit (upper), endocarp *b* cells are missing in the non-explosive *less lignin2* (*lig2*) mutant (middle), demonstrating that these cells are required for fruit to explode. Lignified secondary cell walls are deposited symmetrically in endocarp *b* cells of non-explosive *NST3::VND7* transgenics (lower), demonstrating that the hinged shape of endocarp *b* secondary cell walls is required for explosive seed dispersal.

mechanisms underlying this instantaneous release of stored elastic energy in the fruits of touch-me-not (*I. glandulifera*) and popping cress (*C. hirsuta*) [14,25••]. Fracture is an energy-consuming process, meaning that much of the elastic potential energy stored in fruit walls will be consumed in making cracks, and the remainder will be converted to kinetic energy to launch seeds. Yet Deegan found that almost all the energy stored in *I. glandulifera* fruit walls was converted into kinetic energy [14]. Using a finite element model with the actual geometry of the fruit valve, he showed that 70% of the connecting seam between valves can be cracked without causing explosion [14]. Therefore, due to an optimal shape of the fruit valves, cracks can creep down each connecting seam, priming the fruit for explosion. Explosive fracture of the remaining seams achieves an efficient transfer of elastic to kinetic energy [14]. By contrast to this, the shape of *I. capensis* fruit valves does not allow the seams to precrack [14]. Therefore, explosive seed dispersal in this different touch-me-not species is less energy efficient, resulting in a smaller dispersal distance [23]; associated with a less invasive habit [39].

Geometry is also key to the rapid release of stored energy in *C. hirsuta* — geometry of the fruit valve, but ultimately the geometry of a cell wall in a single cell layer of the valve [25••]. Cells in the inner (endocarp *b*) layer of the valve are asymmetrically thickened by a lignified secondary cell wall on their inner face (red, Figure 3a). Lignin is precisely deposited in these cells to form three stiff rods connected by very thin hinges. The fruit valve needs to coil along its length to reach its relaxed state, but its transverse curvature prevents this (Figure 3a). Opening these hinged cell walls flattens the cross-section of the valve, which removes this energy barrier, allowing the sudden release of elastic energy (Figure 3a). Toy slap bracelets, for example, work by a similar principle. The particular advance in this work came from combining functional and theoretical approaches [25••]. In this case, a mathematical model explicitly described how the geometry of the lignified endocarp *b* cell walls enabled explosive energy release (Figure 3b), and predictions from this model were functionally tested using genetics (Figure 3c). First, *C. hirsuta* mutants with less lignified fruit valves were identified by genome-wide mutagenesis, including a mutant that was missing the endocarp *b* cell layer (Figure 3c). Fruit of this mutant were unable to explode, providing genetic evidence that these lignified cells were necessary for explosive dispersal [25••]. Second, genetically modifying the geometry of these lignified cell walls from hinged to boxed, also rendered the fruit unable to explode (Figure 3c). This provided genetic evidence that the specific geometry of the secondary cell wall was necessary for explosive energy release [25••]. By demonstrating a strict phylogenetic association between explosive seed dispersal and the presence of asymmetric secondary cell wall thickenings in endocarp *b* cells of fruit across the Brassicaceae, the authors concluded, based on combined evidence, that this secondary cell wall pattern was a novelty associated with the evolution of explosive seed dispersal [25••].

Conclusions

Explosive fruit continue to fascinate scientists from diverse disciplines. Considerable progress has been made in recent years by combining approaches from these different disciplines to understand the explosive mechanisms plants use for seed dispersal. These studies have leveraged advances in high-speed imaging, quantitative image analysis [40], and non-destructive methods to measure mechanical properties of cells and tissues [41], thermogenesis [33•], and 3-D microstructure [37]. Combining this quantitative data with state-of-the-art modeling techniques has been a useful approach to identify the mechanical basis of explosive movements [14,25••]. It has also been important to study these complex, biomechanical traits at multiple scales, relating observations at the plant scale all the way down to the cellular and genetic scales, by systematically linking each scale with modeling [25••]. Use of the genome editing tool CRISPR-Cas9 now offers exciting possibilities to combine genetics with biomechanics to study rapid movements in a wide range of plants.

Conflict of interest statement

Nothing declared.

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