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Creating an explosion: Form and function in explosive fruit

Erin Cullen and Angela Hay

Abstract

Adaptations for seed dispersal are found everywhere in nature. However, only a fraction of this diversity is accessible through the study of model organisms. For example, Arabidopsis seeds are released by dehiscent fruit; and although many genes required for dehiscence have been identified, the genetic basis for the vast majority of seed dispersal strategies remains understudied. Explosive fruit generate mechanical forces to launch seeds over a wide area. Recent work indicates that key innovations required for explosive dispersal lie in localised lignin deposition and precise patterns of microtubule-dependent growth in the fruit valves, rather than dehiscence zone structure. These insights come from comparative approaches, which extend the reach of developmental genetics by developing experimental tools in less well-studied species, such as the Arabidopsis relative, *Cardamine hirsuta*.

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Keywords

Explosive seed dispersal, Fruit development, *Cardamine hirsuta*, Lignin, Growth, Cortical microtubules.

Introduction

People are intrigued by explosive fruit and have given evocative names to many plants that disperse their seeds explosively. For example, the Dynamite tree (*Hura crepitans*) [1], Touch-me-not (*Impatiens glandulifera* [2], *Impatiens capensis* [3]), and squirting cucumber (*Ecballium elaterium*) [4]. Dispersal from one location to another is a key part of a plant's life cycle and the only way for a species to change or expand its range. It is not surprisingly then, that plants have evolved diverse mechanisms to disperse their seeds effectively [5]. Many seed dispersal strategies rely on abiotic or biotic dispersal vectors, such as wind, water, or animal agents. For example, the dandelion pappus generates a separated vortex of stably recirculating air to optimise its dispersal by wind [6]. In contrast to this, explosive seed dispersal provides independence from dispersal vectors and relies on mechanical innovations to store and rapidly release elastic energy to launch seeds.

Explosive seed pods typically comprise valves that separate from the fruit at maturity by dehiscence (Figure 1a). While the valves are anchored to the fruit, tension builds up, but cannot be released. This results in mechanical instabilities of the system that increase over time. Once the anchors break, tension is suddenly released, leading to rapid morphing of the valves, causing seeds to shoot out of the pod [7].

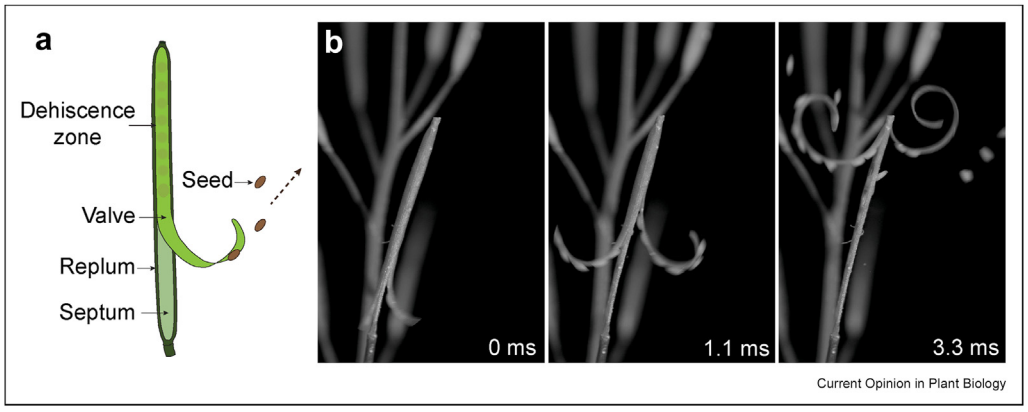
In this review, we highlight recent advances linking form to function in explosive seed pods. *Cardamine hirsuta* is a relative of Arabidopsis that uses an explosive mechanism to disperse its seeds. Comparative studies between these two species have revealed microtubule-dependent growth dynamics and polar lignin deposition by laccases as important innovations underpinning explosive seed dispersal in *C. hirsuta*.

Domesticating seed dispersal

The reduction or elimination of seed dispersal traits was a key step in the domestication of grain crops. For example, wild species and progenitors of cereal and legume crops shed their mature seeds to ensure effective dispersal, while domesticated crops have been selected to retain seeds on the plant to avoid yield loss and to improve efficient harvests [8].

Plants with seed pods, such as the model plant Arabidopsis, typically open at maturity by pod dehiscence to disperse their seeds. The Arabidopsis pod has two valves that encase the seeds. The MADS-box protein FRUITFULL (FUL) specifies valve tissue by restricting the expression of dehiscence zone regulators, such as the basic helix-loop-helix protein INDEHISCENT (IND), to a thin stripe along the valve margin [9,10]. These transcription factors play key roles in a gene regulatory network which ensures that specialised cell

Figure 1



Explosive seed dispersal in *Cardamine hirsuta*. (a) Cartoon of *C. hirsuta* fruit indicating the dehiscence zone separating the valve and replum, and the septum on which seeds lie before being launched by the coiling valve. **(b)** High speed video stills taken at indicated time points in milliseconds (ms). Data from E.C.

types that are required for dehiscence differentiate only at the valve margin.

Unlike Arabidopsis, the valves of wild legumes transform from flat to helical during pod opening. This shape morphing is the result of tension generated in the pod walls as they dry, and causes the pod to shatter upon dehiscence. For example, in the legume *Bauhinia variegata*, the valves have two crossed layers of fibres in the cell wall that are oriented at 45° with respect to the longitudinal axis of the pod [11]. As the flat pod dries, these wall layers contract in orthogonal directions, producing tension that is released upon dehiscence as the valves transform into a helical structure, launching the seeds in the process.

This seed dispersal trait has been selected against during legume domestication. In cultivated soybean (*Glycine max*), the genetic basis of shattering resistance was mapped to *Pdh1*, which encodes a dirigent-like protein [12]. Dirigent proteins are essential for local deposition of lignin in the Casparian strip of Arabidopsis roots [13]. *Pdh1* is highly expressed in the lignified endocarp of the fruit valves and loss of *Pdh1* function reduced the twisting force of the valves, rather than affecting dehiscence zone structure [12]. Hence, natural variants that were selected during soybean domestication did not necessarily correspond to dehiscence genes known from Arabidopsis [8].

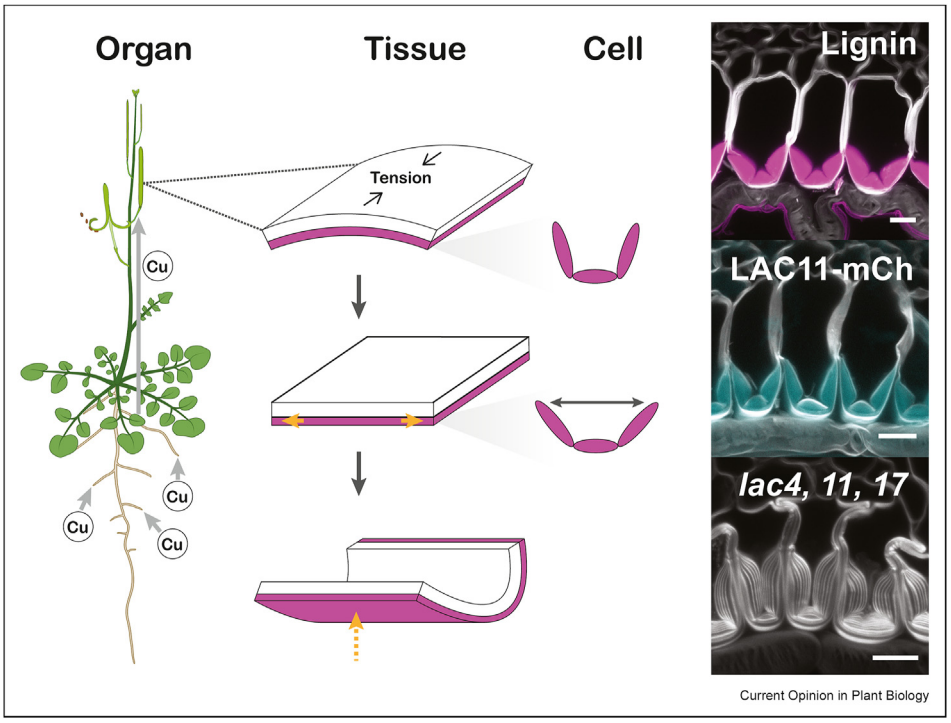
In exploding seed pods of *C. hirsuta*, the two valves change shape from flat to coiled in an ultrafast movement (Figure 1b). This explosive coiling launches seeds at speeds faster than 10 m per second to spread over a metres-wide area [14]. Coiling of the two valves occurs when the pod fractures along its dehiscence zone. Isolating indehiscent mutants in *C. hirsuta* identified conserved functions for *FUL* and *IND* in pod dehiscence [15]. Similar to Arabidopsis, *FUL* is required in *C. hirsuta* to restrict *IND* expression to the valve margin in order for valves to develop correctly. The absence of a dehiscence zone in *ind* mutant fruit prevents the valves from exploding. The tension required to explode is generated in *ind* valves, just as in wild type, but cannot be released unless the valve detaches along the dehiscence zone [15]. Therefore, conservation of the genetic architecture for dehiscent fruit in *C. hirsuta* indicates that the innovations for these seed pods to explode lie elsewhere.

Exploding seed pods

Exploding seed pods evolved in *C. hirsuta* via morphomechanical innovations that allowed the generation and rapid release of tension. A distinctive, polar pattern of lignin deposition within endocarp *b* cell walls was one such innovation (Figure 2). This lignin pattern evolved in association with the trait of explosive seed dispersal in *Cardamine* [14]. Genetic evidence showed that *C. hirsuta* fruit need the endocarp *b* cell layer, and specifically the geometry of its lignified wall (Figure 2), in order to explode [14]. Modeling was used to explicitly describe the mechanics by which ultrafast coiling of the fruit valves was achieved via a sudden change in cell wall geometry that enabled the immediate release of tension via tissue deformations in the valve [14]. Therefore, a combined approach of biology and theory helped to understand the trait at multiple spatial scales from cell to tissue to organ (Figure 2).

What about the scale of genes and molecules? Four lignin-polymerising enzymes — a class III peroxidase (PER66) and three laccases (LAC4, 11, 17) — were recently identified that precisely co-localised with lignin

Figure 2



Form and function of lignified endocarp *b* secondary cell wall in explosive *C. hirsuta* fruit. Cartoon of *C. hirsuta* plant indicating the uptake and translocation of copper (Cu) via the root and shoot to the fruit, which is dependent on the SPL7 pathway. At the tissue scale, tension establishes in the outer exocarp layer (white) of the fruit valve. Upon dehiscence, the valve deforms from curved to flat in cross section by widening (orange arrows) of the endocarp *b* layer (pink). In this conformation, the valve is free to coil explosively (dashed orange arrow) to release valve tension. At the cell scale, the hinged geometry of the lignified endocarp *b* cell walls enable these cells to widen (arrow). LACCASE4, 11 and 17 are necessary for endocarp *b* cell wall lignification and require Cu for their enzymatic activity. Confocal images of wild-type endocarp *b* cells showing lignin stained with basic fuchsin (pink) and LAC11-mCherry protein localisation (cyan) in the secondary cell wall; and the absence of lignin (stained with basic fuchsin) in the endocarp *b* secondary cell wall in *lac4, 11, 17* triple mutants. Cellulose is stained with calcofluor white (shown in greyscale) in all genotypes. Scale bars: 10 μm. Data reproduced from the study by Perez-Anton et al. [16].

in *C. hirsuta* endocarp *b* cells [16] (Figure 2). These oxidative enzymes activate monolignols in the cell wall into radicals that randomly couple to form the lignin polymer. Therefore, the precise localisation of laccases and class III peroxidases in specific cell wall domains can provide a mechanism to control when and where lignin is deposited [17]. However, it is still not well understood how distinct patterns of lignin form in specialised cell types [18], particularly since laccases and peroxidases are encoded by large gene families with high redundancy and pleiotropy. Recently, the stacking of multiple mutants has revealed specific functions for different combinations of these genes in vascular cells [19], the root endodermis [20], and fruit endocarp *b* cells [16]. For example, triple *lac4 11 17* mutants failed to form lignin in the endocarp *b* cell walls of *C. hirsuta* fruit [16] (Figure 2). Yet the additional loss of *PER66* function had no effect [16]. Therefore, it is the three laccases that both co-localise with, and are required for, endocarp *b* lignification. This is reminiscent of the endodermis where five peroxidases were shown to be required to form the Casparian strip, yet loss of nine laccases with endodermal expression had no effect [20]. In both cases, genetics indicated that despite co-localisation, only one class of oxidative enzymes was required for local lignin deposition.

The involvement of laccases in endocarp *b* lignification was discovered through a mutant screen; but not by isolating laccase mutants. Instead, the transcription factor gene *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 7* (*SPL7*) was initially identified by screening for *C. hirsuta* mutants with less lignified fruit valves. Loss of *SPL7* caused a reduction in endocarp *b* lignification and a consequent reduction in how far seeds were dispersed [16]. Intriguingly, the micronutrient copper provides a link between SPL7, laccases and lignin. SPL7 is a conserved regulator of copper homeostasis [21,22] and is both necessary and sufficient for copper to accumulate in the fruit [16]. Since laccases are copper-requiring enzymes, the activity of LAC4, 11 and 17 in endocarp *b* cell walls depends on the SPL7

pathway to ensure sufficient copper for lignin polymerisation [16] (Figure 2). Hence, SPL7 links mineral nutrition to efficient dispersal of the next generation [23].

The distinctive, polar pattern of lignin deposition in endocarp *b* cells is an evolutionary novelty shared by *Cardamine* species. LAC4, 11 and 17 co-localized with this polar pattern in *C. hirsuta*, but adopted a non-polar localisation when these *C. hirsuta* genes were transferred to Arabidopsis, matching the pattern of lignin in Arabidopsis endocarp *b* cells [16]. Therefore, the polar localisation of *C. hirsuta* LAC4, 11 and 17 is not determined by their gene sequences. As these enzymes are glycoproteins, they are likely to be processed in the ER and Golgi and targeted for secretion to precise plasma membrane locations [24,25]; for example, by high densities of microtubules that have been observed at sites of secondary cell wall synthesis [26]. By directing the delivery of components needed to build the secondary cell wall, and guiding the synthesis of cellulose at the plasma membrane, cortical microtubules thus play a critical role in secondary cell wall patterning [27]. These wall patterns, at least in the case of xylem vessels, are 'pre-patterned' at the plasma membrane by small GTPase proteins called Rho of plants (ROPs) [28,29]. ROP signaling pathways induce secondary cell wall patterns through microtubule disassembly [30,31]. Recently, phase separation has been implicated in this process [32], and also a role for microtubule associated proteins in patterning three-dimensional cell wall structures [33]. In summary, the extensive experimental and theoretical work on xylem secondary cell wall patterning [27] provides hypotheses to test in endocarp *b* cells of explosive fruit. Such studies may advance our understanding of how this novel cell wall pattern evolved in *Cardamine*, and also the degree to which secondary cell wall patterning mechanisms are conserved between diverse cell types with specialised functions in different plants.

Harnessing growth to generate tension

The power to explosively coil comes from tension that builds up in the fruit valves of *C. hirsuta*. Rapid coiling results from the differential contraction of valve tissues: the exocarp contracts its reference length while the lignified endocarp *b* resists contraction [14]. Contraction of plant tissues as they dry is a common mechanism to generate tension. For example, the explosive fruit of Chinese witch-hazel *(Hamamelis mollis*) is described as a 'drying squeeze catapult', shooting seeds with a resounding crack, like a fired bullet [34]. By contrast, *C. hirsuta* fruit generate tension while growing, not drying [35].

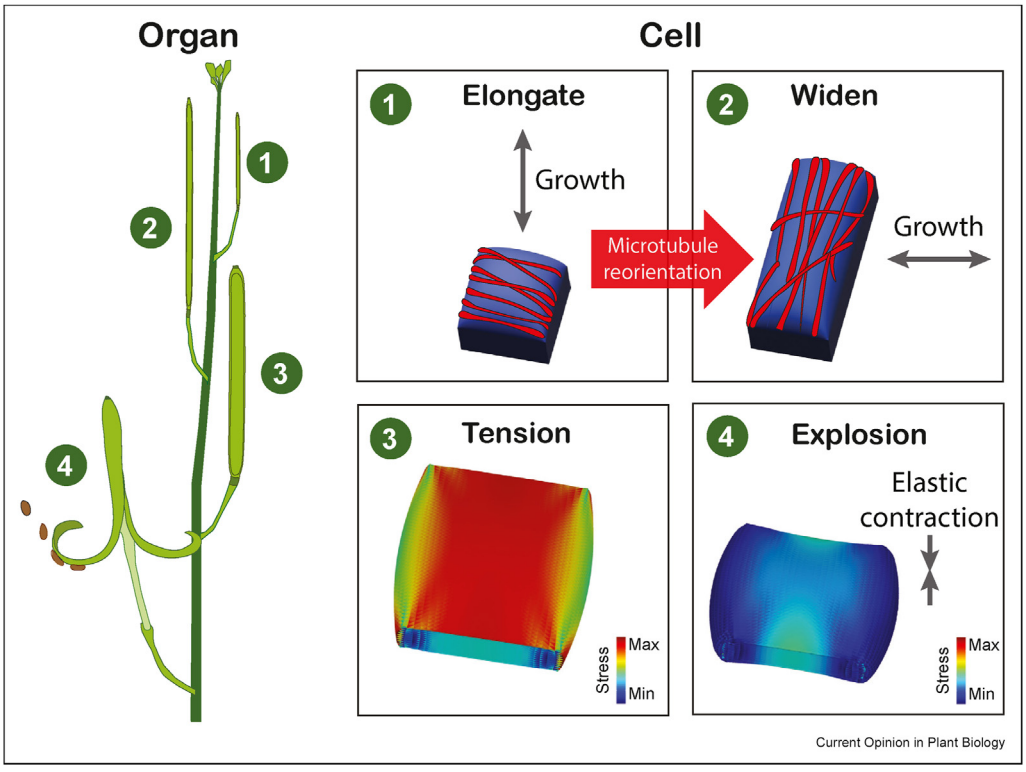
In simple osmotic treatments, exploded valves coiled tightly in pure water and uncoiled in salt solution, showing that valve tissues require turgor to contract and coil [14]. However, turgor-driven growth is a process that relaxes stress in the cell wall [36,37], so it wasn't intuitive how growth could generate tension. Exocarp cell shape, and how it changes through growth, plays a key role in explaining this. Growth of *C. hirsuta* fruit occurs in two phases at both the cell and organ levels: elongation, followed by widening [35] (Figure 3). This switch in growth direction is driven by cortical microtubule reorientation, which reorients the direction of cellulose microfibril synthesis in the wall [35] (Figure 3). As cells widen, their large, square faces, reinforced in the length direction by cellulose, bulge out and accumulate mechanical stress [35] (Figure 3). This curved surface shortens the reference length of each cell, putting the exocarp under tension. Releasing this tension powers explosive coiling by the elastic contraction of each cell to its reference length [35] (Figure 3). In summary, changes to the anisotropic properties of the cell wall, driven by microtubule reorientation, cause exocarp cells to contract their reference length. Tension builds while the growing exocarp tissue is physically anchored to the rest of the fruit.

The fruit valves of Arabidopsis and *C. hirsuta* have a very similar, elongated shape, yet surprisingly, they arise from very different cellular growth patterns [35]. In the case of *C. hirsuta*, this specific pattern of microtubule-dependent growth is a key innovation to generate tension for explosive coiling. This highlights the complex interplay of growth and mechanics during development and the need to quantify growth at cellular scale using live imaging to help address this complexity [38–40].

Seed launch

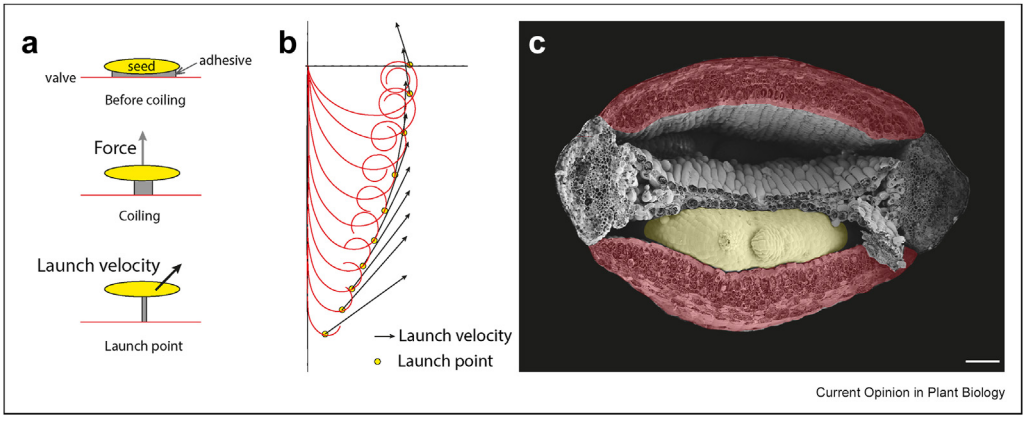
When the valves coil away from the fruit in *C. hirsuta*, the seeds are picked up and remain transiently adhered to the valves until they are launched (Figure 1b). The nature of this adhesion is unknown. One approach to explore how seed launch might work, took advantage of a mathematical model that explained the dynamics of valve coiling [14]. Different hypotheses for seed launch were then compared by matching to data from high-speed videos. The best match was found when the force due to acceleration of the valve was not felt immediately by the seed, but rather integrated over time due to adhesion between the seed and valve [14] (Figure 4a). The force deformed the adhesive, and seeds launched when the adhesive thinned to breaking point (Figure 4a). This meant that seeds launched with a distribution of velocities and angles (Figure 4b). For example, seeds that were released first, launched more perpendicular to the valve axis and with the largest velocity (Figure 4b). While later seeds experienced smaller forces from the coiling valve. These seeds held on to the valve for a longer time before reaching the threshold strain and thus, launched more parallel to the valve axis

Figure 3



Growth leads to tension in *C. hirsuta* fruit. Cartoon of *C. hirsuta* shoot with fruit at different stages of development (left) and exocarp cells of the fruit valve at these same stages (right). (1) During elongation, cortical microtubules (red) are oriented transversely and cells grow in length. (2) Microtubules reorient to the length direction and cells grow in width. (3) Tension develops as the large, square cells bulge out and accumulate stress. (4) To explode, tension is released by the elastic contraction of cell length. Redrawn from the study Mosca et al. [35].

Figure 4



Seed launch involves transient adhesion between valve and seed in *C. hirsuta*. (a) Possible mechanism for seed launch where adhesion (grey) between the valve (red line) and seed (yellow) allows the force of the coiling valve to be integrated over time, stretching the adhesive until breaking point to determine the launch point and velocity of each seed. **(b)** Modeling this mechanism gives different seed launch velocities at different positions along the valve. Arrows indicate the magnitude and direction of seed launch velocity. Yellow dots indicate the point on the valve at which different seeds launch. Redrawn from the study by Hofhuis et al. [14]. **(c)** Cryo-scanning electron micrograph showing a cross sectional view of a mature *C. hirsuta* fruit with two valves (false colored red) and a seed (false colored yellow) clamped between the valve endocarp *a* surface and septum. Attachment between the funiculus and seed was broken during cryo-fracture (see scar next to micropyle). Scale bar: 100 μm. Data from E.C.

and with smaller velocities (Figure 4b). The differential launch velocities of seeds positioned along the valve was sufficient to explain the broad distribution of seeds dispersed by *C. hirsuta* fruit [14].

The transient adhesion between valve and seeds is likely to be influenced by their surfaces, since they are tightly appressed in the fruit before launch (Figure 4c). Adhesive forces that are generated between the seed coat and the valve (endocarp *a* surface) are strong enough to break the umbilical attachment of the funiculus to the seed during launch (Figure 4c). Moreover, this adhesion between the cuticle covering the surfaces of valve and seeds is fully reversible since each seed detaches from the valve as it launches (Figure 1b). A hydrophobic cuticle covers the outer surface of almost all aerial parts of land plants to prevent desiccation. These surface lipids comprise the plant-specific polyester cutin and waxes [41]. The analysis of Arabidopsis mutants has suggested that cuticle nanostructure and chemical composition can influence adhesive forces between adjacent organs; for example, petals in the floral bud [42–45]. Therefore, investigating cuticle function and taking a genetic approach to perturb the continuous surfaces at the valve-seed interface, may yield interesting insights into seed launch in *C. hirsuta*.

Conclusions and outlook

Understudied organs provide a valuable approach to learn through diversity. Extending our knowledge of seed dispersal strategies beyond Arabidopsis is likely to inform rational breeding approaches to prevent crop yield loss from pod shatter. Explosive seed pods evolved in *C. hirsuta* through innovations that connect form and function. The specific cell shape acquired through growth in the exocarp, and the particular geometry of the polar, lignified endocarp *b* cell wall, have key biomechanical functions in the generation and rapid release of tension. Identifying copper-dependent laccases that control this lignin deposition, and the SPL7 transcription factor required to ensure copper homeostasis, highlights how mineral nutrition can be linked to seed dispersal.

Further insights into explosive seed dispersal may come from extending comparative studies to additional *Cardamine* species. The amphicarpic plant *Cardamine chenopodiifolia* has a particularly fascinating seed dispersal strategy. These plants develop aerial fruit that disperse their seeds explosively, but they also develop non-explosive fruit from underground flowers that self-pollinate and set seed below ground. Resources such as a draft genome, reference transcriptome and plant transformation in *C. chenopodiifolia* [46,47] pave the way to explore further aspects of under-studied organs and discover new biological mechanisms.

Declaration of competing interest

The authors declare the following financial interests/personal relationships that may be considered as potential competing interests: Erin Cullen reports financial support was provided by Deutsche Forschungsgemeinschaft Walter Benjamin postdoctoral fellowship. Angela Hay reports financial support was provided by Deutsche Forschungsgemeinschaft FOR2581 Plant Morphodynamics grant.

Data availability

No data was used for the research described in the article.

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** of outstanding interest

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