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PROTOCOL NOTE

A maceration technique for soft plant tissue without hazardous chemicals

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Abstract

Premise: Current methods for maceration of plant tissue use hazardous chemicals. The new method described here improves the safety of dissection and maceration of soft plant tissues for microscopic imaging by using the harmless enzyme pectinase.

Methods and Results: Leaf material from a variety of land plants was obtained from living plants and dried herbarium specimens. Concentrations of aqueous pectinase and soaking schedules were optimized, and tissues were manually dissected while submerged in fresh solution following a soaking period. Most leaves required 2–4 h of soaking; however, delicate leaves could be macerated after 30 min while tougher leaves required 12 h to 3 days of soaking. Staining techniques can also be used with this method, and permanent or semi-permanent slides can be prepared. The epidermis, vascular tissue, and individual cells were imaged at magnifications of 10× to 400×. Only basic safety precautions were needed.

Conclusions: This pectinase method is a cost-effective and safe way to obtain images of epidermal peels, separated tissues, or isolated cells from a wide range of plant taxa.

KEYWORDS

cell isolation, dissection, epidermal peel, maceration, pectinase

The unobstructed observation of plant cells with objective lens microscopy is a useful tool for botanical education and is critical for the acquisition of anatomical data. The use of histological techniques to remove obstacles can improve microscopic image quality. If material is not being sectioned, techniques including epidermal peels, careful dissection to expose specific tissue regions, and isolation of cells can help achieve clear microscopic images. In the case of taxonomically significant leaf surface features, images can be obtained at a high cost by scanning electron microscopy (SEM) or at a fraction of the cost through epidermal peels (Bussotti and Grossoni, 1997; García-Gutiérrez et al., 2020; Leandro et al., 2020). When morphometric data pertaining to individual cells is desired, the plant tissue must be dissected without damaging the integrity of the cell shape. There are modern methods that use advanced tools such as lasers to achieve microdissections or internal imaging without dissection like X-ray computed tomography (Millar et al., 2015; Piovesan et al., 2021), but these techniques are expensive and not feasible for engaging students in the classroom. For the purpose of this paper, we refer to any dissection that relies on the separation of tissues at a cellular level, including different scales of deterioration, as maceration.

A list of previously published techniques for plant tissue maceration, along with their reaction agents and approximate cost for making 100 mL of solution, are provided in Appendix 1. These publications collectively tackle a range of purposes for maceration in plant science, but the list is not exhaustive. While these methods all share the aim of disrupting the adhesion of cells, some have been developed solely for use in one taxon (Jain, 1976; Rajendra et al., 1977; Segatto et al., 2004), and others involve the use of hazardous or expensive chemicals (Pohl, 1967; Gouse and Yunus, 1972; Mohan Ram and Nayyar, 1977). The earliest maceration techniques are often associated with woody tissues and were used to facilitate the visualization and interpretation of vessel elements, tracheids, fibers, and other cell types (Jeffrey, 1917; Tupper-Carey and

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Priestley, 1924; Franklin, 1945). Wood tissue macerations are still regularly used in plant anatomy classes, but students usually only view purchased slides because hazardous chemicals are involved in the separation of these heavily lignified tissues. Maceration techniques for soft plant tissues, which rely on weaker dilutions of the chemicals, may produce excellent images, but still involve risks unsuitable for classrooms (Gouse and Yunus, 1972; Mohan Ram and Nayyar, 1974; von Konrat et al., 1999). An alternative method that employs pectic enzymes for soft tissue macerations was proposed by Hohl (1948) based on her experiences working in food technologies. At the time this method was developed, pectic enzymes were a safer option that showed promise for producing microscopic images of cells and organelles in a range of land plants, and these enzymes were beginning to be more widely commercially available (Brown, 1951; Chayen, 1952; Orgell, 1955). Ironically, these methods were developed before there was a full "understanding of the chemical structure of the 'intercellular cement' between plant cells" (Bateman, 1968) and were pioneered for research on cellular connections. In a trade-off between safety and image quality, acid buffers can be used to optimize enzymatic reactions of pectinase at a pH range of 3.3 to 5.3 depending on the plant substrate (Sato, 1968; Lendzian et al., 1986). Other methods include pectinase alongside a suite of dangerous reagents to break apart plant tissues (Xie et al., 2022).

Primary walls denote borders between distinct plant cells, and the middle lamella is an intercellular layer between the walls of adjacent cells that cements the cells together (Esau, 1977). It is composed primarily of pectin, a group of polysaccharide chains, and degradation of this layer results in plant cells that can be easily dislodged and separated. Various pectinolytic enzymes, often known as pectinases, react with the polysaccharides of the middle lamella through hydrolysis (Yadav et al., 2009). Originally evolved by fungi for the predation of plant tissues, pectic enzymes are now industrially extracted from fungi for a variety of applications in the food industry (Kashyap et al., 2001; Jayani et al., 2005). We developed a technique that uses pectinase to image undamaged mesophyll cells for three-dimensional shape analysis, and expanded the process to loosen cells, aid dissection, and perform macerations to obtain microscopic images in a wide range of plants. We tested the ability of pectinase to work in a purely aqueous solution on a range of plants. Given the relative safety and cost effectiveness of this technique, we suggest that it can be used for a variety of applications ranging from educational activities to use in research involving plant anatomy.

METHODS AND RESULTS

We chose to work with a commercially available pectic enzyme, as it was relatively cheap, available in reasonable quantities, and shipped as a shelf-stable dry powder (LD Carlson Company, Kent, Ohio, USA). We purchased 450 g of pectic enzyme for $8.50 USD, which equates to $0.22 per 100 mL of maceration solution. Pectinase powder is shelf-stable indefinitely and, in liquid form, is viable for up to a year if refrigerated. The Safety Data Sheet (SDS) for the product describes the composition as including active ingredients polygalacturonase, pectinesterase, and pectolyase with an inert maltodextrin carrier. The SDS lists inhalation of the dry powder as the primary hazard, but these pectic enzymes are safe to humans after being mixed into water. This is supported by the Food and Drug Administration's approval of these enzymes for use in wine and beer making (Food and Drug Administration, 2022). The package instructions suggest a ~1:1500 ratio of pectinase powder to water (or fruit juice), but we found that the aqueous pectinase solution was easily adjustable and that higher concentrations improve maceration in some leaves.

TABLE 1 Taxa with a range of leaf qualities used to demonstrate pectinase maceration.

Material type	Leaf characteristic Delicate	Average	Tough
Fresh material	Euphorbiaceae	Asteraceae	Ericaceae
	Euphorbia L. 'Starblast Snowdrift'	*Helianthus annuus* L. 'Teddy Bear'	*Rhododendron* L. PJM Group
	[latex]	[trichomes]	[coriaceous]
Fresh material	Malvaceae	Typhaceae	Crassulaceae
	Gossypium barbadense L.	*Typha angustifolia* L.	*Sempervivum* cf. *tectorum* L.
	[trichomes]	[thick cuticle]	[thick cuticle]
Fresh material	Aspleniaceae	Poaceae	Pinaceae
	Thelypteris dentata (Forssk.) E. P. St. John	*Phragmites australis* (Cav.) Trin. ex Steud.	*Pinus sylvestris* L.
			[thick cuticle]
Herbarium specimen	Aspleniaceae	Poaceae	Pinaceae
	Athyrium filix-femina (L.) Roth	*Phragmites australis* (Cav.) Trin. ex Steud.	*Pinus sylvestris* L.
			[thick cuticle]

We used leaf material from 10 land plants with broad systematic relationships and a range of leaf qualities (Table 1). Thin, delicate leaves pose a challenge for maceration because they can too readily break down. Conversely, resistance to maceration might be expected in thick, tough leaves. After considering these challenges, we sampled from plants that were immediately available and used leaves exhibiting extreme delicateness ($n = 3$) and toughness ($n = 3$). All of the "delicate" leaves were chartaceous, but thin and easily torn. The "tough" leaves were either coriaceous or thick and succulent. We also included plants ($n = 3$) with leaves representing average leaf durability that required some effort to tear and were chartaceous or spongy. We included plants with leaves with hairs ($n = 2$) and thick waxy cuticles ($n = 3$) that could be problematic for microscopic imaging. We also tested the ability to use dry plant material from herbarium specimens (Appendix 2), by macerating tissue obtained from oven-dried voucher specimens in combination with conspecific, fresh tissue obtained from living plants. The plant materials used in this study are presented in Appendix 3. Material was removed from living plants and herbarium specimens, cut into segments 2–4 cm long by 1–2 cm wide, and submerged in aqueous pectinase solution. We experimented with different concentrations of pectinase solution and different soaking times. Pectinase as a maceration tool is adaptable for a range of imaging projects and can be designed for taxon-specific leaf qualities (Appendix 2).

The presoaking stage allows the pectinase to loosen the connections between cells of the epidermis and those adjacent to cut edges. If the aim of the maceration is to obtain images of epidermis features, degradation of this tissue can be prevented by reducing or skipping the presoaking time. Some leaves are delicate enough that maceration on fresh material can be conducted using high concentrations of pectinase with little or no presoaking. To macerate internal tissues, incisions can be made to improve infiltration. We conducted the majority of macerations using a standard aqueous pectinase solution of 12 g (or 4 tablespoons) of pectinase powder in 100 mL of distilled H_2O (recipes and instructions provided in Appendix 2). A stronger concentration was used in some cases with tough leaves, but clumping of enzymes and reduced visibility of the solution prevented further enzymatic saturation. Average leaves benefited from 2–4 h of presoaking, and tough leaves required >24 h of presoaking to break apart as desired. Care is needed, however, as plant tissues allowed to soak in aqueous pectinase solution for multiple days may begin to decompose, and mold growth may form on the liquid surface.

We used Nikon DS-Vi1 cameras (Nikon Instruments, Melville, New York, USA) mounted on a dissecting microscope (Nikon SMZ 745 T) or a compound microscope (Nikon Eclipse 55i) to capture microscopic images (Figure 1A–L). In most leaves, the maceration can proceed under considerable control until the desired amount of disintegration is achieved. We describe here several examples from our study material. In the process of exposing vascular patterns in *Euphorbia* L. 'Starblast Snowdrift' (Figure 1A), the mesophyll cells are cleared away, allowing pectinase to reach new regions of tissue. For an epidermal peel using pectinase, the process involves tissue removal of everything except a chosen epidermis. Removal of only trichomes is possible without damaging other surface features, as demonstrated with the *Helianthus annuus* L. 'Teddy Bear' petiole (Figure 1B). The contrast between the coriaceous epidermis and the thin mesophyll layer created challenges for determining the enzyme concentration, soaking times, and force during dissection in the *Rhododendron* L. PJM Group leaf (Figure 1C). These decisions are shaped by the target of the study (see Appendix 2C). A stoma of a *Gossypium barbadense* L. leaf (Figure 1D) was photographed after removal of the mesophyll cells to increase light on the subject. When the study pertains to the internal anatomy, the epidermis can be removed in strips, as was done to expose the aerenchyma of the *Typha angustifolia* L. leaf (Figure 1E). Revealing the intact cell walls of aerenchyma could be an improvement to viewing a prepared cross-section where aerenchyma could be misinterpreted as gaps between cells. In the succulent *Sempervivum* cf. *tectorum* L. (Figure 1F), we also focused on the interior of the leaf by imaging intact mesophyll cells. Scraping away the cuticle and epidermis was too aggressive for the *Sempervivum* mesophyll, causing the cells to rupture. Instead, the leaf should be carefully cut to expose mesophyll on sharp corners of tissue. Pectic degradation is aided by the high surface area to volume ratio in this case, and intact cells prominently positioned on the prepared piece of leaf material can be gently loosened.

Because fume hoods are not needed and personal protective equipment is minimal, cells that have been removed with pectinase can be rotated or positioned properly with a tool under the microscope immediately before imaging. For example, the epidermal cells of the *Thelypteris dentata* (Forssk.) E. P. St. John leaf (Figure 1G) could be dislodged with a jet of solution or a gentle nudge from a probe; this exploration of plant anatomy could be an asset to a plant anatomy course. The process of macerating the leaf tissue can be just as informative as the resulting image. The strategy of removing all tissue except the epidermis worked well in the *Phragmites australis* (Cav.) Trin. ex Steud. leaf (Figure 1H). Easier macerations were possible when whole sections of tissue could be removed, as was seen when the section of epidermis was removed from the *Pinus sylvestris* L. leaf (Figure 1I). The fresh material was pliable and this aided the dislodging of intact cells. The herbarium specimens used during this study presented different qualities and results. The delicate and desiccated *Athyrium filix-femina* (L.) Roth leaf fractured in geometric lines, instead of separating at the cell wall borders (Figure 1J). The strategy of removing all tissue except the epidermis worked well in *Phragmites australis*, with the images of fresh material (Figure 1H) being comparable to images obtained from herbarium material (Figure 1K). Working with dried leaf material was not problematic in this scenario, because the task during maceration was to destroy and remove cells, while the tissue of interest remains intact and can be imaged. Although the *Pinus sylvestris* herbarium specimen was collected and dried 100 years ago, the leaf cells taken from this material acted as a reasonable substrate (Figure 1L).

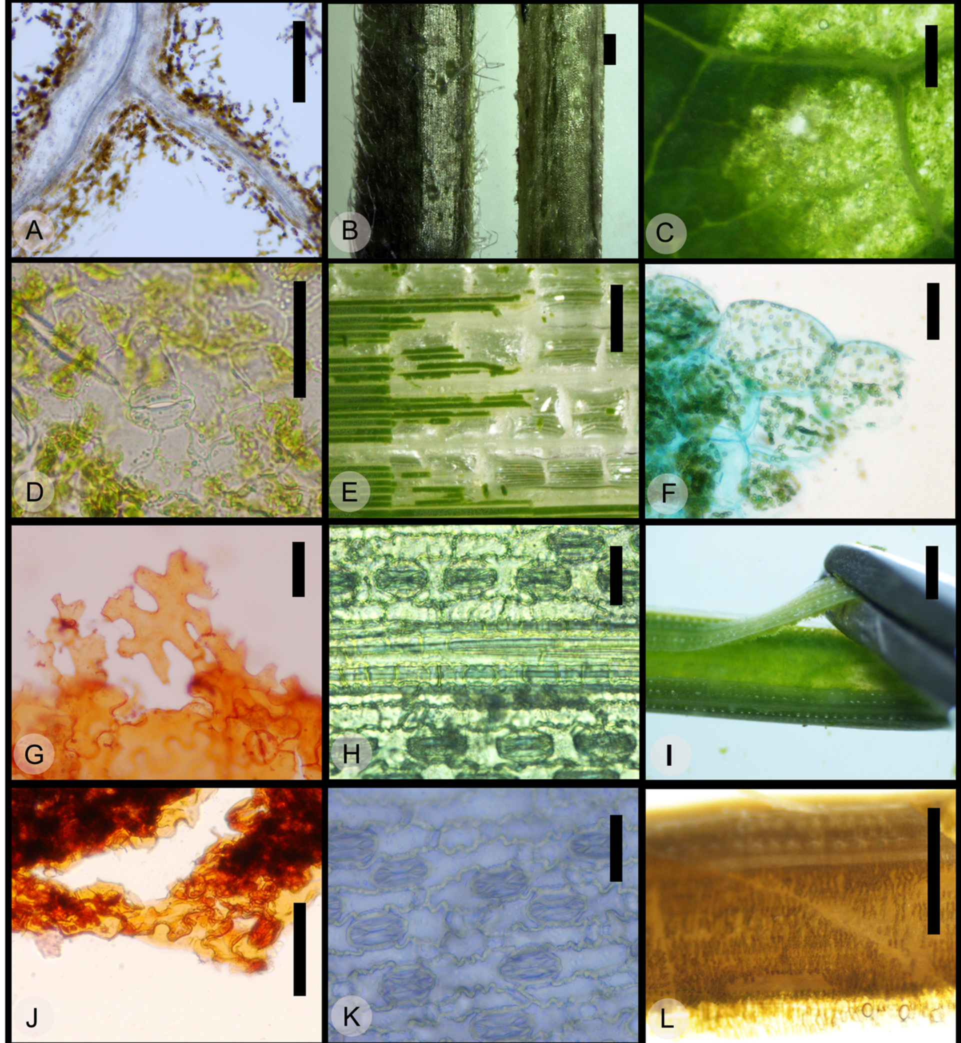

FIGURE 1 Microscopic images of leaf anatomy after maceration with pectinase. (A) Vascular bundles of *Euphorbia* 'Starblast Snowdrift'. Scale bar = 100 μm. (B) Petioles of *Helianthus annuus* 'Teddy Bear' with (left) and without (right) trichomes. Scale bar = 1000 μm. (C) Epidermal peel in progress showing vasculature in *Rhododendron* PJM Group. Scale bar = 200 μm. (D) Epidermis of *Gossypium barbadense*. Scale bar = 50 μm. (E) Aerenchyma of *Typha angustifolia* exposed after removal of epidermis. Scale bar = 1000 μm. (F) Mesophyll cells of *Sempervivum* cf. *tectorum*. Scale bar = 100 μm. (G) Epidermal cells of *Thelypteris dentata*. Scale bar = 50 μm. (H) Stomata and silica cells from fresh material of *Phragmites australis*. Scale bar = 50 μm. (I) Epidermal layer from fresh material of *Pinus sylvestris* being removed from leaf with a tweezer. Scale bar = 400 μm. (J) Epidermal cells of *Athyrium filix-femina* from herbarium specimen. Scale bar = 50 μm. (K) Stomata and silica cells of *Phragmites australis* from herbarium specimen. Scale bar = 50 μm. (L) Mesophyll exposed after removal of epidermis of *Pinus sylvestris* from herbarium specimen. Scale bar = 400 μm.

CONCLUSIONS

We set out to obtain microscopic images using only pectinase as a maceration aid. A variety of taxa were selected to impose challenges to visualization and illustrate that pectinase as a maceration agent in aqueous solution should work on any land plant. Thick cuticles inhibited infiltration, but upon exposure during dissection, pectinase was able to liberate mesophyll cells. Maceration of thick leaves is aided by making incisions in the epidermis to allow the pectinase solution into the mesophyll. We found that maceration of thin leaves can be problematic because they can quickly tear apart. The removal of cells was easier in thick leaves because there is a visible region of mesophyll after the epidermal cells have been removed, the intact epidermis can provide some resistance for dissection, and the multicellular depth of the mesophyll allows dissection to move gradually through the leaf. The maceration of parallel-veined monocot tissue was found to be simpler than the maceration of reticulate-veined dicot tissue due to the relative ease of removal of parallel sections of tissue. Similar cell patterning assisted the removal of epidermal tissue in *Pinus sylvestris* (Figure 1I). However, in *Gossypium barbadense*, clusters of mesophyll cells constituting whole areoles within the reticulate venation could dislodge together.

This maceration technique may be used as a quick and simple way to observe leaf epidermal micromorphology to determine whether SEM imaging could potentially be advantageous and even used as a substitute for SEM imaging

when features such as dense epicuticular wax or highly branched papillae obscure underlying stomatal complexes or other features such as silica bodies. As this maceration technique can liberate individual cells without causing damage to the cell wall itself, it is ideal for the analysis of cell shapes in three dimensions. The relative cost efficiency and safety of this method could facilitate large surveys of plant collections to identify potentially synapomorphic or functional characters. We have used leaves as an example of soft plant tissue, but this method could also be used for stems, fruits, flowers, and roots with additional time given to soak tissues in the pectinase solution. Dyes and stains can be included, but will introduce their own hazards. Similarly, it would be difficult and potentially detrimental to subject the final product of pectic maceration to dehydration baths for mounting or staining. As maceration using pectinase involves minimal hazards to human health, is relatively cheap, and requires only readily available enzymatic materials, it presents a simple and safe way to observe three-dimensional cell shapes or plant tissue layers in both the classroom setting and the research laboratory.

AUTHOR CONTRIBUTIONS

P.C.K. and L.G.C. planned the research. P.C.K., E.K.M., and J.J.N. conducted the research. E.K.M. vouchered specimens. All authors contributed to the writing of the manuscript and its revision. All authors approved the final version of the manuscript.

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DATA AVAILABILITY STATEMENT

All supporting data is published within the article.

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APPENDIX 1. Previously published methods for achieving maceration of soft plant tissue.

Intended purpose	Specialized equipment/chemicals	Estimated cost (USD) per 100 mL	Reference
Wood vascular tissue maceration	Nitric acid, chromic acid	$9.52	Jeffrey (1917)
Root maceration	Ammonia	$5.50	Tupper-Carey and Priestley (1924)
Wood maceration	Glacial acetic acid, hydrogen peroxide	$5.00	Franklin (1945)
Petiole and fruit maceration	Pectic enzyme	$0.22	Hohl (1948)
Nuclei isolation	Pectinase, hydrochloric acid, aceto-carmine	$23.72	Brown (1951)
Root maceration	Pectinase	$0.22	Chayen (1952)
Cuticle isolation	Pectic enzyme, acetic acid	$5.22	Orgell (1955)
Fruit and leaf maceration	Ethylenediaminetetraacetic acid (EDTA)	$53.25	Letham (1960)
Maceration for cell counts	Cellulase, citric acid, disodium hydrogen phosphate	$2.82	Rijven and Warlaw (1966)
View leaf epidermis in Poaceae	Hot nitric acid, potassium chlorate, potassium hydroxide	$9.52	Pohl (1967)
View leaf epidermis in gymnosperms	Hot nitric acid	$9.52	Gouse and Yunus (1972)
View leaf cuticles	Scanning electron microscope, chromium trioxide	$6.89	Alvin and Boulter (1974)
View leaf epidermis	Cupric sulphate, hydrochloric acid	$2.38	Mohan Ram and Nayyar (1974)
View leaf epidermis in conifers	Hydrogen peroxide, acetic acid	$5.70	Jain (1976)
View leaf epidermis	Perchloric acid	$22.71	Mohan Ram and Nayyar (1977)
Wood vascular tissue maceration	Sonicator, nitric acid, chromic acid	$7.00	Schmid (1982)
View leaf epidermis in Poaceae	Nail polish	$7.54	Hilu and Randall (1984)
Cuticle isolation in conifer leaves	Pectinase, sodium azide	$14.34	Lendzian et al. (1986)
Bryophyte capsule cell layer visualization	Pectinase, lactic acid	$6.14	von Konrat et al. (1999)
Leaf clearing	Glacial acetic acid, hydrogen peroxide	$5.92	García-Gutiérrez et al. (2020)
Fruit vascular tissue maceration	Scanning electron microscope, sodium hydroxide, acid fuchsin, ethanol, chloral hydrate, pectinase, cellulase, hydrogen peroxide, glacial acetic acid, dipotassium hydrogen phosphate	$3.14	Xie et al. (2022)

APPENDIX 2. Detailed protocol instructions for pectinase maceration techniques.

A. Supplies

Equipment	Purpose
Pectinase	Enzymes for the maceration of plant tissue that break pectin chains of the middle lamella, allowing separation of cells. See note in **H. Recipes** at the end of this appendix about the optimal pH and temperature of pectinase.
Water	The solvent in which pectinase is dissolved to form a solution. It is preferable to use distilled H_2O (dH_2O).
Beaker	250- or 500-mL beaker to mix and store pectinase solution. Approximately 50 mL of pectinase solution per leaf and 200 mL of pectinase for each dissecting station.
Scale or measuring spoons	Recipes are provided in grams and tablespoons (tbsp.).
Storage containers	The plant material should be completely submerged in pectinase solution and stored in an airtight container for the duration of the presoaking period.
Dissecting microscope	This equipment is for a dissection station. The slide will sit in the Petri dish, creating a base on which the plant material can be macerated. The Petri dish will be filled with pectinase solution so that the plant material and slide are submerged.
Petri dish	
Microscope slides	
Weights	Weights can be helpful for holding down plant material during the dissection and imaging if tissue needs to remain submerged in the Petri dish. They may also be used to hold down cover slips.
Forceps	These tools can be used to manipulate and dissect the plant tissue. Individuals should experiment and find a combination of tools that enables them to comfortably interact with the cells. In some cases, removal of cells can be achieved using tools that do not actually touch the cells, but by swishing the aqueous pectinase solution over a region of plant material. The waves will provide fresh pectinase and dislodge cells.
Pipette	
Paintbrush	
Probe	
Dissecting razor	
Stains or dyes	These are optional and can be included at any stage of the process. Good results were obtained with Fast Green added in early steps to visualize the dissection and the subsequent images. Gloves and additional safety precautions should be considered depending on the stains or dyes used.
Mounting adhesive	For permanent or semi-permanent preservation of the slide. Mounting adhesive should be aqueous or water soluble.
Cover slips	

B. Collection of material

Fresh material: If you are using fresh plant material, avoid letting it dry out or wilt. Either begin the maceration process immediately or place the tissue in an air-tight container (plastic bag or food storage container) along with a damp paper towel until maceration. Consider using scissors as a safe way to collect plant material.

Dry material: If you are using plant tissue from herbarium specimens or other desiccated sources, it is advisable to rehydrate the tissue before attempting pectinase maceration. Water alone will not soak into the plant tissue quickly enough, so rehydrating involves submerging the plant tissue (10–50 min) in a solution that can permeate the dried plant cells, making the tissue pliable again. A recipe for Pohl's solution (Pohl, 1965), which accomplishes this task, is included at the end of this appendix.

C. Planning

Decide on the desired outcome (e.g., epidermal peel, cellular isolation) to determine what cells and/or tissue will be removed. This will determine how long to soak and the appropriate concentration of pectinase during different stages. Pectinase concentrations could be higher in classroom demonstrations to achieve a goal quickly, but lower concentrations and a slower pace may produce better histological preparations for permanent collections. The table below can guide you on appropriate pectinase concentrations and soaking times depending on plant tissue attributes and the specific cells you wish to image.

Concentrations were made with weak, standard, and strong ratios of pectinase to water with instructions for each provided below in **Recipes**.

Leaf attribute	Epidermal peel	Tissue separation	Cell isolation
Soft	PC = weak	PC = weak, standard	PC = standard
	PT = 30 min	PT = 0.5–4 h	PT = 1–4 h
	MC = standard	MC = standard	MC = standard, strong
Average	PC = standard	PC = standard	PC = standard
	PT = 2–4 h	PT = 2–4 h	PT = 2–12 h
	MC = standard	MC = standard	MC = standard
Tough	PC = standard	PC = standard	PC = standard, strong
	PT = 4–12 h	PT = 4–48 h	PT = 4–48 h
	MC = strong	MC = standard	MC = strong

Note: PC = presoaking concentration; PT = presoaking time; MC = maceration concentration.

Cut material: Pectinase will interact with the edges and surfaces of the plant material first. Some strategies may take advantage of cutting the material into many small pieces to increase the exposed surface area, but many of the maceration techniques will benefit from a section of plant tissue 2–4 cm long by 1–2 cm wide. This shape allows stabilization under the dissection microscope by providing a side to hold down while working on the other half.

Storage: We have found that disposable, polypropylene 50-mL centrifuge tubes work well for storage during the soaking stage. Other airtight containers (e.g., food storage containers, Petri dishes) may work, but the plant tissue should be submerged, and larger containers will require more pectinase solution. Storage containers should be labeled with the following information: the type of plant material, the pectinase concentration, and the date and time at which soaking was initiated.

D. Presoaking

Before manual maceration, it is advantageous to let the plant tissue soak in pectinase solution. This stage begins to separate the cells of the epidermis and will soften the connections between internal cells near cut edges. If the plant material is left submerged in pectinase but otherwise undisturbed for multiple days, the tissue will become extremely soft. Plant material may appear to remain intact, but efforts to remove it from the solution will promote disintegration. Eventually, cells will lyse and only lignified cells like vessel elements will remain.

E. Manual dissection

Hints: As the maceration occurs, new regions of cells will be exposed to pectinase. Patience and intermittent breaks, allowing more hydrolysis, can lead to great results. Holding the tissue down with a finger or a weight can stabilize and provide resistance for maceration. Light scraping, pressure washing with jets of liquid, and simple forceps removal of undesired tissue are the primary techniques. Use and revisit different techniques throughout the process to dislodge cells.

Epidermal peel: If the maceration is being done to remove adaxial, mesophyll, and vascular tissue to facilitate imaging of the abaxial epidermis (or reversed for the adaxial epidermis), it may be helpful to secure the desired epidermis first. This is not necessary for all plants, but in cases when leaf tissue can disintegrate too quickly, we recommend combining the pectinase maceration approach with the nail polish method to obtain images of epidermis features (Hilu and Randall, 1984). Nail polish can be applied to the desired epidermis and allowed to dry. This will bind the exposed epidermal cells together. The leaf tissue can then be placed with the nail polish and desired epidermis down on a microscope slide in a Petri dish. The tissue should be submerged in a high-concentration pectinase solution, and undesired cells can be removed by maceration. Complete maceration may leave just the nail polish mold, but if you take care as you move deeper through the leaf, the cells of the desired epidermis can be left intact.

Tissue isolation: If the maceration is being done to expose a specific region of the plant material, work can occur at or near the edges. Plant tissue should be placed on a microscope slide in a Petri dish and submerged in a high-concentration pectinase solution. Vascular bundles will provide more resistance for removal, but in some cases can be useful in removing other tissues along with it.

Individual cells: If the maceration is being done for teaching purposes and the desire is for cells to only be viewed live through the dissecting scope, then the slide in the Petri dish can be covered with a high-concentration pectinase solution. Physical agitation with any of the dissection tools will result in cells wiggling, loosening, and moving. As the tissue separates, cells may come off individually or in clumps.

If the maceration is being done to produce isolated cells on a slide to be kept long term, then the final slide should not be in a Petri dish. Prepare the tissue, if necessary, by dissecting toward the desired tissue region in a Petri dish on a slide submerged in pectinase solution. If, for example, you are interested in mesophyll cells, remove some epidermis first. The maceration can be continued by transferring the tissue to a fresh slide under the dissecting microscope without a Petri dish. A small amount of pectinase solution can be added with a pipette where you intend to macerate the tissue. This step will be similar to preparing a wet mount slide, with the bubbles and tissue visible through the dissecting scope before a cover slip is added. A pipette of fresh, high-concentration pectinase solution should be kept close by and used to prevent desiccation of the tissue. Scrape the desired cells onto the slide and then remove larger clumps of debris. A small drying period to reduce the amount of liquid on the slide may help provide room for mounting solution before a cover slip is added.

F. Mounting

This maceration method uses water to make an aqueous pectinase solution. We used an aqueous mounting solution for permanent or semi-permanent slide preparation. Gelatin/glycerin could provide another suitable mounting option. It may be difficult to conduct ethanol dehydration utilizing a permanent mounting medium, such as Permount, because the already macerated tissues may be lost in the process.

G. Imaging and analysis

Microscopic imaging can be accomplished through the dissecting scope at 4–10× magnification with the material still on the slide in the Petri dish. Lighting and reflections may be difficult to maneuver because the material will still be submerged. A wet mount can be made of the macerations because the tissue should remain in aqueous solution through the entire process. In some cases, flushing the macerated tissue with jets of water from a pipette can remove undesired cellular debris before mounting.

Revisit planning after completing the "Imaging and Analysis" stage, and try different concentrations to improve taxon-specific methodologies. The cheap and safe nature of pectinase as a maceration tool promotes experimentation and allows mistakes to be made.

H. Recipes and notes

Recipe for modified Pohl's solution for rehydrating dry material
300 mL dH_2O
100 mL 1-Propanol
2 mL dish soap

Recipe for 1% Fast Green
200 mL 95% ethanol
2 g Fast Green
Allow to mix for 24 h
Filter through a coffee filter prior to use

Recipe for weak aqueous pectinase solution
100 mL dH_2O
6 g (or 2 tbsp.) pectinase

Recipe for standard aqueous pectinase solution
100 mL dH_2O
12 g (or 4 tbsp.) pectinase

Recipe for strong aqueous pectinase solution
100 mL dH_2O
15 g (or 5 tbsp.) pectinase

Optimal temperature and pH for pectinase reaction

A maximum rate of separation with pectinase can be achieved at pH 5.3 (see Sato, 1968), but the pectolytic activity of the enzymes is enough to obtain desired results with tap water or dH_2O.

Room temperature (20–23°C) is suitable for macerating plant tissue with pectinase.

APPENDIX 3. Plant materials. All specimens are deposited at the Ada Hayden Herbarium at Iowa State University (ISC), Ames, Iowa, USA. Information presented: species name and authority, herbarium accession number, collector names, collection number, collection location, collection date.

Athyrium filix-femina (L.) Roth, ISC 404273, collected by: D. A. Gualls and C. L. Johnson-Groh, No. 1606, Dolliver State Park. Webster Co., Iowa, 25 May 1985

Euphorbia L. 'Starblast Snowdrift', ISC 456575, collected by E. K. McMurchie, No. 1568, United States, Iowa, Story County, Iowa State University. Richard Pohl Conservatory at Bessey Hall. Cultivated, 4 April 2022

Gossypium barbadense L., ISC 456572, collected by E. K. McMurchie, No. 1573, United States, Iowa, Story County, Iowa State University. Richard Pohl Conservatory at Bessey Hall. Cultivated, 5 April 2022

Helianthus annuus L. 'Teddy Bear', ISC 456571, collected by E. K. McMurchie, No. 1567, United States, Iowa, Story County, Iowa State University. Richard Pohl Conservatory at Bessey Hall. Cultivated, 4 April 2022

Phragmites australis (Cav.) Trin. ex Steud., ISC 264850, collected by: C. P. Malone, No. 362, Audubon Co., Iowa, 3 July 1967

Pinus sylvestris L., ISC 31505, collected by: L. H. Pammel, State Center, Iowa, 26 Aug 1902

Rhododendron L., PJM Group, ISC 456574, collected by E. K. McMurchie, No. 1571, United States, Iowa, Story County, Iowa State University. Lagomarcino Hall courtyard. 42.029666°N, −93.645323°W, 296 m. Cultivated, 4 April 2022

Sempervivum cf. *tectorum* L., ISC 456570, collected by E. K. McMurchie, No. 1572, United States, Iowa, Story County, Iowa State University. Richard Pohl Conservatory at Bessey Hall. Cultivated, 4 April 2022

Thelypteris dentata (Forssk.) E. P. St. John, ISC 456573, collected by E. K. McMurchie, No. 1569, United States, Iowa, Story County, Iowa State University. Richard Pohl Conservatory at Bessey Hall. Cultivated, 4 April 2022

Typha angustifolia L., ISC 456576, collected by E. K. McMurchie, No. 1570, United States, Iowa, Story County, Iowa State University. Richard Pohl Conservatory at Bessey Hall. Cultivated, 4 April 2022