Vegetative propagation and ex situ conservation of Chapman's rhododendron in Atlanta Botanical Garden

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Abstract

Rhododendron chapmanii is a federally endangered, Florida-endemic shrub with a highly restricted and fragmented distribution. Updated field surveys reveal steep population declines across its range due to habitat loss, fire suppression, hurricane damage and over-collection. In response, the Atlanta Botanical Garden (ABG) has implemented an integrated conservation strategy that combines in situ monitoring, demographic research and comprehensive ex situ safeguarding. Conventional hardwood and semi-hardwood cuttings achieved 70–80% rooting success when propagated by ABG's conservation horticulture team. A new, efficient micropropagation protocol was developed using Anderson's rhododendron medium supplemented with zeatin and indole-3-butyric acid, enabling rapid, genetically stable shoot proliferation and high rooting rates. These propagation techniques support efforts to expand the species' safeguarded genetic base, reduce pressure on wild populations and provide high-quality plant material for planned reintroductions guided by species-distribution modelling. This integrated approach positions ABG as a key leader in the recovery of *R. chapmanii*, demonstrating the critical role of coordinated propagation, genetic safeguarding and habitat management in preventing extinction of narrowly endemic plants.

Introduction

Rhododendron chapmanii (Alph. Wood) A. Gray, commonly known as Chapman's rhododendron, is a perennial evergreen shrub endemic to northern Florida, USA. The species occupies an extremely narrow geographic range and occurs naturally at low population density, making it intrinsically vulnerable to environmental and anthropogenic pressures. First described by Gray in 1877 (Gray, 1877), it

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was later treated as a variety of *R. minus* – *R. minus* var. *chapmanii* – by Duncan & Pullen (1962), Luteyn *et al.* (1996) and Gandhi & Zarucchi (2009). However, morphological distinctiveness, biogeographical isolation and updated floristic treatments support the recognition of Chapman's rhododendron as a distinct species (Miller, 2013; Weakley & Southeastern Flora Team, 2025). Consistent with the most recent United States Fish and Wildlife Service five-year status review (USFWS, 2024), we here follow this treatment as a separate species.

Chapman's rhododendron has been federally listed as Endangered since 1979 and is currently ranked G1 (Critically Imperilled) by NatureServe Explorer and Critically Endangered (CR) on the Red List of Rhododendron (Gibbs *et al.*, 2011). Only four primary population centres are currently known – located in Gulf, Clay, Liberty and Gadsden counties, Florida. A single flowering plant with approximately 15 stems was newly discovered in Walton County in 2022, extending the known range to the north-west (USFWS, 2024). Ongoing population genetic analyses indicate that the Hosford population (Liberty and Gadsden counties) and Gulf County population are genetically distinct, whereas additional sampling and analysis of the Clay and Walton County populations are under way to characterise population genetic structure across the species range. Chapman's rhododendron plays an important role in the local ecosystem as a persistent resprouting understorey shrub in fire-dependent communities, and as an early spring floral resource for native pollinators (USFWS, 2024). The species faces multiple ongoing threats from urban development, timber harvesting, agricultural conversion, inadequate fire management and hurricane impacts. The historically largest population near Hosford (Liberty County) has suffered extensive decline following clearing and conversion of private land to cattle pasture. Surveys conducted by ABG in 2022 documented surviving plants in only two of the five element occurrences (EOs) at the privately owned land in Hosford, each with fewer than 50 individuals, an alarming reduction from the 2,942 clumps recorded in 1997 (USFWS, 2024).

The Camp Blanding population in Clay County, first documented in 1942 with more than 60 clumps (Totten & Major, 1944), persists today as only 31 clumps on protected land (USFWS, 2024). In contrast, the Gulf County population is now the largest and most stable, with 811 clumps across eight EOs extending from Mexico Beach into St Joseph Bay State Buffer Preserve (SJBSBP). Active management and prescribed fire at SJBSBP have helped to stabilise and modestly increase population numbers there (USFWS, 2024). Despite legal protection, the horticultural appeal of the species makes it vulnerable to over-collection (Gensel & Blazich, 1985; USFWS, 2024). As the only native evergreen rhododendron in Florida, it is valued for its early blooms and heat tolerance for breeding. The species is a seed parent for several registered cultivars including 'April Pink', 'Pink Magic', 'Southland', 'Chapmanii Wonder', 'Gable's Early Bird' and 'Bowie'. It is a pollen parent in 'Huggy Bear' and 'Pink Elegance' (Leslie, 2004; American Rhododendron Society, 2026).

ABG leads coordinated actions for ex situ safeguarding and conservation of Chapman's rhododendron, which is recognised as a priority species within its Southeastern Center for Conservation programmes. ABG botanists in Florida initiated a detailed demographic study in 2021 at SJBSBP – one of the species' key strongholds – to inform

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adaptive habitat management and guide future reintroduction within its historical range. Complementing these field efforts, the conservation horticulture team successfully propagated Chapman's rhododendron from cuttings to establish genetically verified conservation collections. ABG monitors each known wild population and collaborates with land managers on habitat stewardship, particularly the implementation of prescribed fire, which is essential for maintaining the species' fire-dependent pineland habitat. At its Conservation Safeguarding Nursery in Gainesville, Georgia, ABG currently cultivates 49 wild-origin accessions, ensuring living backups of wild genotypes. Since 2023, the Micropropagation Laboratory has developed an efficient protocol for in vitro shoot multiplication and rooting, preserving tissues under controlled conditions and supplying plantlets for ex situ cultivation and research. Building upon these findings and ex situ propagation efforts, ABG is now developing plans to reintroduce additional populations within the species' natural range to enhance its long-term viability and resilience. Together, these integrated strategies – linking field monitoring, propagation and genetic safeguarding – embody ABG's broader mission to conserve imperilled native plants through science-based propagation and collaborative recovery planning across the south-eastern United States.

In vitro propagation and conservation

Micropropagation is widely used not only for the mass propagation of commercial ornamental rhododendrons and azaleas, but increasingly for the conservation of threatened species and wild relatives of horticultural cultivars. Over 95 taxa of rhododendron – including species, subspecies, varieties and cultivars – have been studied for in vitro propagation (Tan & Go, 2022). Heritage, wild-collected, rare and unusual rhododendron taxa represented by only a few individuals have been successfully micropropagated in the Royal Botanic Garden Edinburgh to preserve genetic material and expand living collections (Davidson, 2019). Given that the genus *Rhododendron* includes more than 1,000 species worldwide, in vitro protocols are often species-specific, as physiological responses to media and plant growth regulators vary widely.

For Chapman's rhododendron, an early micropropagation protocol was successful using shoot-tip cultures on Woody Plant Medium supplemented with a high concentration (10 mg/L) of the cytokinin 6-(γ,γ-dimethylallylamino) purine (2-iP) (Blazich *et al.*, 1986). Although this treatment induced both axillary and adventitious shoot formation, the elevated cytokinin level caused genetic instability in adventitious shoots and poor elongation of regenerated shoots. These limitations highlighted the need for optimised cytokinin balances and culture conditions to achieve stable, elongated shoots suitable for rooting and acclimatisation.

We developed an improved and efficient micropropagation protocol based on the axillary shoot proliferation method previously optimised for *Vaccinium arboreum* (Li *et al.*, 2021), thereby minimising the risk of somaclonal variation. Anderson's rhododendron medium (AN) (Anderson, 1978, 1980) – S816, purchased from PhytoTech Labs, Lenexa, Kansas – was used as the basal medium and supplemented with zeatin (ZT) and indole-3-butyric acid (IBA). Solutions of 30 g/L sucrose and 6.5 g/L agar were added, and pH was adjusted to 5.2 before autoclave.

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Explant preparation

Current-year semi-hardwood shoots (5–10 cm) were collected from healthy stock plants and used as explant material. After removing all leaves, stems were cut into 1–2 cm segments, each bearing at least one node. The nodal segments were washed thoroughly with a mild hand-soap solution and rinsed under running tap water for 30 minutes, then surface sterilised in a laminar-flow cabinet. The sterilisation protocol included sequential immersion in 70% (v/v) ethanol for 10 seconds, 0.1% (w/v) mercuric chloride for 15 minutes (with gentle agitation), followed by three rinses with sterile distilled water.

Culture initiation and multiplication

Twenty sterilised nodal segments were placed on initiation media in 25 mm x 150 mm polycarbonate test tubes, one nodal segment per test tube. Anderson's rhododendron medium supplemented with 0.05 mg/L IBA and 0.5 mg/L of one of three cytokinins – ZT, BA (6-Benzylaminopurine), or 2-iP – was compared. Axillary buds emerged within two weeks on uncontaminated segments (Fig. 1A). Among the treatments, AN + 0.5 mg/L ZT + 0.05 mg/L IBA performed best, with a 25% success rate. Shoots elongated to 1–2 cm after 2–3 weeks and were excised and transferred to a multiplication medium (AN + 1.0 mg/L ZT + 0.01 mg/L IBA, Fig. 1B). Each shoot produced 5–10 new axillary shoots within five weeks (Fig. 1C). When the axillary shoots numbered 30, media with three different concentration combinations were compared for best proliferation (AN + 0.1 mg/L ZT + 0.01 mg/L IBA; AN + 0.5 mg/L ZT + 0.05 mg/L IBA; AN + 1.0 mg/L ZT + 0.05 mg/L IBA). Three replications were performed with ten shoots in each 220 mL glass culture jar filled with 30 mL media. After several monthly subcultures on the same medium, shoot proliferation on AN + 0.5 mg/L ZT + 0.05 mg/L IBA remained vigorous, stable and morphologically uniform without hyperhydricity (Fig. 1D). The multiplication rate was on average about ten shoots per passage, and most new shoots developed from the original stem (Fig. 1E). Any adventitious shoots arising from the basal callus were discarded during subculture to reduce the potential for genetic variation.

Rooting

Elongated shoots (>4 cm) were transferred to the rooting medium in 473 mL (16 oz) deli containers, 20 shoots per container. Rooting media of half-strength AN supplemented with 1 g/L activated charcoal and 0, 0.5 and 1.0 mg/L IBA were compared, five containers of each IBA concentration. Shoots in media with 1.0 mg/L IBA formed roots in vitro within three weeks with 100% rooting success, with most roots emerging directly from the stem and minimal callus formation (Fig. 1F).

Acclimatisation and germplasm conservation

Rooted in vitro shoots were gently washed off to remove the culture medium and transplanted into 72-cell plug trays (54 × 28 × 4.5 cm; individual cell volume ≈ 38 mL) filled with a peat-based potting substrate (PRO-MIX, Premier Horticulture Ltd) without additional fertiliser (Fig. 2A). The trays were placed inside a humidity dome to maintain high relative humidity during initial acclimatisation (Fig. 2B). Within four weeks, the plantlets developed functional, fibrous root systems that fully occupied the plug cells (Fig. 2C). The well-rooted liners were then transplanted into 10-cm pots containing an aged pine-bark-based potting mix fertilised

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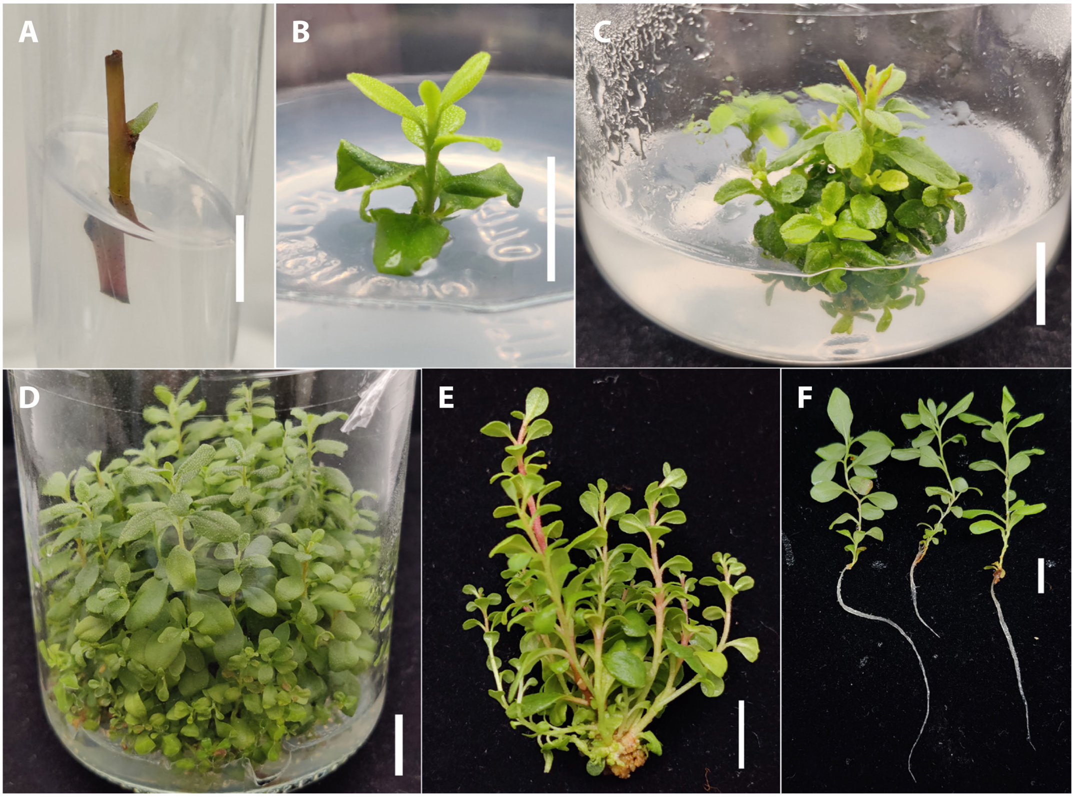

Fig. 1 The in vitro propagation process of Chapman's rhododendron. **A** New bud emerged from the initial nodal segment. **B** New emerged shoots 1–2 cm long were excised from initial nodal segment and cultured on shoot multiplication medium. **C** New shoots proliferated. **D** Shoot proliferation after several generations of subculture. **E** Clump of proliferated shoots from a single explant. **F** In vitro rooted shoots. Bars=1 cm. Photos: Q. Li.

with 5 g Osmocote slow-release fertiliser (19:5:8) per pot and grown under standard greenhouse conditions (Fig. 3). The plants reached up to 35 cm in height after growing in 16-cm pots for six months (Fig. 4A) and 60 cm with multiple shoots after 11 months (Fig. 4B).

Using this protocol, 11 wild-origin accessions of Chapman's rhododendron were successfully established in vitro beginning in July 2025. These accessions represent genetic material rescued from a natural population in Florida on privately owned land slated for commercial development, ensuring the preservation of its germplasm for future restoration and conservation research. More materials will be micropropagated from our previously collected plants from different populations in order to provide sufficient plants for reintroduction with increased genetic diversity.

Plant tissue culture serves as a sophisticated bridge between field collection and long-term preservation. Tissue culture technologies can asexually propagate large numbers of uniform plantlets, which promotes the protection, reintroduction and restoration of species in cases in which traditional propagation is not adequate (Kulak *et al.*, 2022). The in vitro techniques can store plant germplasm in aseptic culture conditions, but can also provide easy access for the evaluation, utilisation and safe exchange of plant material (Coelho *et al.*,

Fig. 2 Acclimatisation of shoots. **A** Rooted in vitro plantlets transplanted in 72-cell plug trays filled with peat-based potting mix. **B** Plug tray kept in a humidity dome to maintain air humidity for acclimatisation. **C** Root ball well formed in the plug showing fibrous roots six weeks after acclimatisation. Photos: Q. Li.

2020). In vitro materials also make long-term viable storage of plant germplasm possible through cryopreservation methods (Chandran *et al.*, 2023).

Propagation by cuttings

Chapman's rhododendron can be propagated by both hardwood and semi-hardwood cuttings through treatment with a 5,000–10,000 ppm IBA solution or 8,000 ppm dry powder (Gensel & Blazich, 1985). Building on this research, ABG's conservation horticulture team has refined and implemented standardised propagation protocols to expand ex situ safeguarding collections. These protocols incorporate wounding of basal cuttings, controlled misting and bottom heat to optimise rooting efficiency and survival rates across diverse wild genotypes. Since 2020, ABG has propagated Chapman's rhododendron by conventional stem cuttings on three separate occasions, enabling comparison of propagation performance across seasons and specific treatments.

Trial 1 – Hardwood cuttings in autumn (2020)

The first propagation trial used hardwood cuttings collected on 27 October 2020 from 40 wild-origin individuals and processed at ABG's Conservation Greenhouse on 3 November 2020. Leaves were removed from

Fig. 3 Acclimatised plantlets grown in 10-cm pots filled with aged pine-bark-based potting mix. Photo: Q. Li.

the lower 60% of each cutting, and flower buds were removed from half the samples to evaluate potential effects on rooting performance. Basal ends were re-cut at a 45° angle and wounded on one side along the lower 1 cm of the stem to promote adventitious root initiation. Cuttings were treated with a 2-second dip in a 1:1 dilution of Dip 'N Grow® liquid rooting hormone, resulting in a final solution containing 5,000 ppm IBA and 2,500 ppm 1-naphthaleneacetic acid.

Cuttings were inserted 4 cm deep into a rooting substrate composed of 1:1 (v/v) peat moss and medium-grade perlite. Maternal lines were maintained separately in labelled nursery pots and placed on a bottom-heated mist bench set to 22 °C. Ambient greenhouse temperatures fluctuated from approximately 10 °C at night to 22 °C during the day. Misting was programmed for 30 seconds every 30 minutes during daylight hours through winter; as photoperiod and temperature increased, the frequency was gradually increased to 30 seconds every 15 minutes.

Well-developed roots formed within 150 days after striking the cuttings. The overall success rate across the 40 accessions was 70%, although results varied among maternal lines, with four lines yielding no rooted cuttings. Rooted plants were transferred into a high-drainage greenhouse substrate on 15 April 2021. By spring 2022, plants had grown large enough for pot-upgrading and were flowering heavily.

Trial 2 – Hardwood cuttings in spring (2021)

A second batch of hardwood cuttings was collected from 18 wild-origin individuals on

Fig. 4 Tissue culture plants grown in a 16-cm pot **A** after 6 months; **B** after 11 months. Photos: Q. Li.

4 March 2021 and processed on 10 March 2021. Two modifications were introduced relative to the first trial: (1) the hormone dip duration was increased from 2 seconds to 4 seconds, and (2) the rooting substrate was altered to a mixture of 1 part milled sphagnum, 1 part coarse perlite and 4 parts medium perlite (by volume). All other treatments remained consistent with Trial 1, aside from the naturally rising ambient temperatures of spring.

This trial produced an overall rooting success of 80%, with noticeably faster root initiation and development. Rooted cuttings were ready for potting after 90 days, in contrast to the 150 days required for the autumn batch. As in Trial 1, rooting success was again varied among maternal lines, suggesting the presence of genotype-dependent or maternal plant status differences in propagation performance.

Trial 3 – Semi-hardwood cuttings in summer (2025)

The third propagation trial employed semi-hardwood cuttings collected on 22 July 2025 and processed the following day. In this trial, the hormone dip duration was increased to 5 seconds. Based on observations from earlier trials, in which basal wounding rarely stimulated callus or root formation, the longitudinal wound was omitted. Instead, a light surface scrape was applied with a razor blade held at a 90° angle to the stem, taking care not to break the epidermis. The misting regime was also substantially modified for summer conditions: mist was applied for 15 seconds every 8 minutes until late October, after which the cycle was reduced to 12 seconds as the temperature and light level declined seasonally.

The first visible roots were observed on 28 August 2025, indicating a more rapid initiation compared with previous propagation cycles. However, as of 11 November 2025, most cuttings had not yet produced sufficiently developed root systems or new shoot growth for transplanting (Fig. 5). This was probably influenced by seasonal reductions in day length and light intensity, which can restrict carbohydrate availability and root elongation.

Comparative outcomes and integration with ex situ safeguarding

Across the three cutting-propagation trials, Chapman's rhododendron exhibited strong potential for vegetative propagation under controlled greenhouse conditions, though success varied by season and maternal line. Hardwood cuttings taken in autumn and early spring achieved the highest and most consistent rooting percentages (70–80%), while semi-hardwood cuttings collected in midsummer exhibited faster root initiation but slower subsequent root system development, probably reflecting seasonal differences in carbohydrate allocation, photoperiod and physiological activity. Overall, these results suggest that late-winter and early-spring hardwood cuttings provide the most effective balance of rooting success and root system quality. The refinement of conventional cutting techniques, complemented with micropropagation, enables the maintenance and expansion of genetically verified wild-origin accessions. Together, these propagation approaches strengthen ex situ safeguarding capacity, supply plant material for restoration trials, and support research-based horticultural practices within the broader conservation and recovery strategy for Chapman's rhododendron.

Fig. 5 Vigorous roots four months after cuttings taken in July 2025. Photo: Q. Li.

To date, 165 individual plants have been successfully propagated from 71 distinct maternal lines representing three wild populations. Of these, 49 individuals are now established in-ground at ABG's Conservation

Safeguarding Nursery. This collection serves as a genetically verified living repository. It provides essential material for research and restoration. ABG intends to continue its development so that it can contribute to long-term ex situ conservation of this federally endangered species.

Ex situ conservation in the Conservation Safeguarding Nursery

The ex situ conservation activities carried out in the Conservation Safeguarding Nursery provide a genetically diverse safety net beyond native habitats and are integrated into the strategy of Botanic Gardens Conservation International's Global Conservation Consortium for *Rhododendron*. Through the efforts of ABG's conservation horticulture team, each accession has been clonally propagated at least twice, establishing a redundancy plan that preserves genetic diversity should any individual plant be lost.

In spring 2023, one clone of each of the 49 accessions was planted into the permanent ex situ safeguarding conservation orchard. This orchard is a wooded, north-facing slope that was formerly a *Pinus strobus* (white pine) plantation. Decades of pine silviculture resulted in a deep, acidic organic layer, creating ideal soil conditions for Chapman's rhododendron and other acidophilic species. Prior to planting out in 2023, selective removal of remaining white pine opened the canopy, leaving a hardwood-dominated overstorey of *Quercus alba*, *Liriodendron tulipifera*, *Oxydendron arboreum*, *Prunus serotina* and *Acer rubrum*. This management action increased light penetration and produced partial shade conditions comparable to the species' natural habitat. During the first two years after planting, the plants were irrigated weekly by a drip irrigation system; in 2026 irrigation shifted to a supplemental watering schedule as the plants have reached full establishment. The plants were well established and in full bloom one year after being planted (Fig. 6). To prevent unintended seedling recruitment and preserve the genetic integrity of the collection, the plants are routinely deadheaded so that no seed is produced within the orchard. There are plans to expand the propagation and planting efforts so that three clones of each accession are permanently established in the conservation orchard, further enhancing genetic representation and long-term safeguards for Chapman's rhododendron.

Conclusion

ABG serves as a critical conservation partner for Chapman's rhododendron, applying its botanical expertise, advanced propagation capabilities and long-established safeguarding infrastructure to support the species' long-term survival amid environmental threats. Through its integrated conservation pipeline, ABG employs both vegetative propagation by cuttings and micropropagation to maintain and expand a genetically diverse collection of wild-origin accessions in ex situ care. Reliable and scalable propagation methods enable the production of genetically representative plant material suitable for future reintroductions throughout its historical range, including at extirpated sites identified by recent species-distribution modelling (FNAI, 2022). The DNA samples from different populations are also deposited in the ABG's genetic lab, where the analysis of genome-wide diversity, hybridisation and potential inbreeding depression is ongoing to inform conservation strategies.

Fig. 6 Chapman's rhododendron in full bloom in April 2024, one year after being planted in the conservation orchard at the Conservation Safeguarding Nursery. Photo: T. Biggers.

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Coordinated under Botanic Gardens Conservation International's Global Conservation Consortium for *Rhododendron*, ABG's efforts directly advance progress towards the recovery criteria for this federally endangered species. In addition, in vitro conservation provides an efficient means of preserving a broader array of wild genotypes within the limited space typical of botanic garden facilities. The highly efficient micropropagation protocol developed for Chapman's rhododendron not only secures valuable germplasm but also supplies substantial numbers of plants suitable for horticultural use, thereby reducing collecting pressure on wild populations. Together, these strategies position ABG to play a leading role in the comprehensive recovery and long-term resilience of Chapman's rhododendron.

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