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Seed germination reports for Sicilian endemic taxa of the genus *Helichrysum* (Asteraceae)

Abstract

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In the present study, successful germination protocols for 11 different taxa of the genus *Helichrysum* native to Sicily are discussed. Eight of them are representative of strict endemic Sicilian taxa, 2 are Italian sub endemic and one, namely *H. stoechas*, is a widespread W Mediterranean species. Given the controversial taxonomic status and relationships of the Mediterranean *Helichrysum*, the taxonomic approach enhancing the existing diversity of Sicilian populations was herein adopted from current literature. First germination records are given for some of the investigated taxa.

Key words: germination protocols, Italian flora, native plants, seeds, Sicily.

Introduction

Helichrysum Mill. is a large and heterogenous genus of the sunflower family (Asteraceae), currently consisting of ca. 600 worldwide distributed species. The taxonomic and phylogenetic relationships within this genus in many ways still remain controversial and not satisfactorily resolved (Galbany-Casals & al. 2004, 2014).

As far as the Italian territory is concerned, the existing populations reveal a high rate of morphological polymorphism with some constant features often linked to strict geographical distribution and limited environmental niches (Brullo & Guarino 2018). Pignatti (1982) originally reported 11 taxa altogether (6 species plus 5 varieties). Later, Conti & al. (2005) listed 13 taxa, represented by 11 different species with 4 subspecies. Other floristic and taxonomic studies (Aghababyan & al. 2007; Giardina & al. 2007) discriminated against 15 different taxa, with 11 species and 4 subspecies, while Greuter (2006) listed only 11 taxa, namely consisting of 8 species with 5 subspecies. Phylogenetic analyses based on both morphological and molecular data (Galbany-Casals & al. 2004, 2006, 2014; Herrando-Moraira & al. 2017) recognised only 7 taxa for the Italian flora, all belonging to the section *Stoechadina* (DC.) Gren. & Godr. Additionally, contemporary interspecific hybridization is known to occur in the genus *Helichrysum* (Jeanmonod 1996; Galbany-

Casals & al. 2006), thus further complicating the taxonomic framework. More recently, Bartolucci & al. (2018) accepted for the Italian flora 12 taxa, corresponding to 7 different species and 5 subspecies, with most of the Sicilian endemic taxa treated as synonyms of *H. pendulum* (C. Presl) C. Presl in accordance with the cited phylogenetic studies. Lastly, Brullo & Guarino (2018) distinguished 14 different species with 8 subspecies, gathered in 3 main groups based on some peculiar morphological characters. Here, we applied the latter classification of the Italian *Helichrysum* since it better reflects the diversity of the Sicilian investigated populations, additionally indicating into brackets accepted names from Greuter (2006) when different.

In this work, we present successful germination protocols for eleven taxa of *Helichrysum*, nine of which are strictly endemic to Sicily and the surrounding archipelagos. All germination tests were carried out at the Catania Germplasm Bank using four constant temperature regimes (10, 15, 20 and 25°C) and both light and dark conditions.

75. *Helichrysum archimedeum* C. Brullo & Brullo ex Greuter (Asteraceae)

Accession data

Si: Sicily. Mts. Iblei, Ferla (Siracusa) (WGS84: 37.119167°N, 14.945834°E), calcareous rocks, 540 m a.s.l., 17 Jun 2013, S. Brullo (UNICT_086, BGS-CT, Catania Germplasm Bank).

Germination data

Pre-treatments: sterilization with a 1% sodium hypochlorite water solution for 3 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 5 ml of sterilized distilled water.

Sample size: 100 seeds for each test (25 × 4 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T ₁ [d]	T _{s0} [d]	T _{max} [d]	MTG [d]
90%	constant 10°C	12/12h	9.0	13.4	32.0	15.5
90%	constant 15°C	12/12h	5.0	10.9	32.0	13.2
89%	constant 10°C	0/24h	12.0	15.4	32.0	17.5
85%	constant 20°C	12/12h	5.0	14.0	32.0	15.0
84%	constant 15°C	0/24h	5.0	9.7	32.0	11.5

Observations

Helichrysum archimedeum is a suffruticose chamaephyte endemic to the Iblei Mts. in south-eastern Sicily, where it is a member of the casmophytic vegetation (*Dianthion rupicolae*) growing on the calcareous slopes of Hyblaean canyons. Seeds of *H. archimedeum*

germinated in the thermal range between 10 and 20°C, reaching the highest germination rates (90%) at 10 and 15°C under light conditions. Increasing temperature caused a significant drop in seed germination, which was only 47% at 25°C and 12/12h photoperiod. The light seems to positively affect seed germinations, because tests carried out under full darkness gave lower results with the increase in temperature, with 50% of germination at 20°C and just 9% at 25°C. As predictable, lower temperature (10°C) resulted in the slowest germination speed especially under full darkness, with a germination delay shifting from 5 to 9-12 days. Royal Botanic Gardens Kew (2021) reported for this species (provenance not specified) a germination percentage of 98% and 92% at 15°C and 20°C, respectively, under a 12/12h photoperiod, while 90% was reached with the alternating temperature 25/10°C.

76. *Helichrysum errerae* Tineo (Asteraceae)

Accession data

Si: Sicily. Pantelleria (Trapani) (WGS84: 36.73750°N, 12.001112°E), volcanic debris and basalt outcrops, 120 m a.s.l., 11 Jun 2014, *S. Brullo & L. Scuderi* (UNICT_134, BGS-CT, Catania Germplasm Bank).

Germination data

Pre-treatments: sterilization with a 1% sodium hypochlorite water solution for 3 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 5 ml of sterilized distilled water.

Sample size: 100 seeds for each test (25 × 4 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T ₁ [d]	T ₅₀ [d]	T _{max} [d]	MTG [d]
99.0%	constant 15°C	12/12h	5.0	8.3	21.0	10.1
97.0%	constant 20°C	12/12h	3.0	7.7	30.0	9.8
96.0%	constant 10°C	12/12h	9.0	14.5	30.0	16.4
96.0%	constant 10°C	0/24h	9.0	10.4	22.0	12.1
93.0%	constant 15°C	0/24h	5.0	6.4	22.0	8.4
80.0%	constant 20°C	0/24h	5.0	9.7	29.0	12.2

Observations

Helichrysum errerae is another strict Sicilian endemism, confined to the island of Pantelleria, where it is the main member of the *Matthiola pulchellae-Helichrysum errerae* pulvinous community occurring between the internal part of the coastal cliffs and the

inland vegetation all along the island. As the previous species, it also showed the highest germination rates (99-96%) in the thermal range between 10 and 20°C under a 12/12h photoperiod, with optimal results (99%) at 15°C. The seeds were not light sensitive at the lowest temperatures, reaching comparable germination percentages both in light and total dark conditions. At the highest temperature (25°C) the germination rate fell to 67% with a 12/12 h photoperiod, dropping down to 30% under dark conditions. These results somehow agree with Royal Botanic Gardens Kew (2021), reporting for this species a 100% of germination at 20°C and 12/12h photoperiod, while the alternating thermoperiod 25/10°C under light conditions gave 88% of germinated seeds.

77. *Helichrysum hyblaeum* Brullo [=? *H. hyblaeum*] (Asteraceae)

Accession data

Si: Sicily. Gela (Caltanissetta), Passo delle Pantanelle (WGS84: 37.047810°N, 14.386846°E), xeric steppe grasslands on limestone, 120 m a.s.l., 20 Jun 2005, S. Sciandrello (BGS-CT/SS109, Catania Germplasm Bank).

Germination data

Pre-treatments: sterilization with a 1% sodium hypochlorite water solution for 3 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 5 ml of sterilized distilled water.

Sample size: 100 seeds for each test (25 × 4 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T ₁ [d]	T ₅₀ [d]	T _{max} [d]	MTG [d]
80.0%	constant 15°C	12/12h	5.0	7.0	26.0	8.9

Observations

Helichrysum hyblaeum is circumscribed to south-eastern Sicily, where it grows in the xeric grasslands of the Hyblean Plateau on calcareous and marly slopes. This is the first germination report for this taxon. The highest germination percentage (80%) was obtained at 20°C and 12/12h photoperiod. The other tested temperature regimes provided similar results, with germination percentages just a little lower (78% and 76% at 20 and 25°C, respectively), except for 10°C which provided only 47% of germinated seeds. Seeds did not show a photoperiod sensitivity, because germination percentages were comparable at both light and dark conditions, except at 10°C where the full darkness improved seed germination to 68%.

78. *Helichrysum italicum* subsp. *siculum* (Jord. & Fourr.) Galbany, L. Sáez & Benedí

Accession data

Si: Sicily. Mt. Etna, Nicolosi (Catania) (WGS84: 37.650556°N, 14.988889°E), volcanic rocks, 1060 m a.s.l., 26 Jul 2013, *S. Bogdanovic, C. Brullo, S. Brullo & G. Giusso* (UNICT_087, BGS-CT Catania Germplasm Bank).

Germination data

Pre-treatments: sterilization with a 1% sodium hypochlorite water solution for 3 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 5 ml of sterilized distilled water.

Sample size: 100 seeds for each test (25 × 4 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T ₁ [d]	T ₅₀ [d]	T _{max} [d]	MTG [d]
85.0%	constant 15°C	0/24h	5.0	7.4	22.0	9.8

Observations

This is a sub endemic taxon, also reported for Calabria (S Italy) and Tunisia, which grows both on calcareous and volcanic substrata, in different habitats, ranging from sea cliffs to sand dunes, rocky places and even roadsides, in a wide altitudinal range (0-1330 m a.s.l.). The investigated population from Mt. Etna reached 85% of germination at 15°C under full dark conditions, while the other tested temperatures (10 and 20°C) provided lower results (77% and 66% respectively), falling to no more than 50% under light conditions. High temperature (25°C) strongly affected seed germination which ranged between 18% and 29% in 12/12h photoperiod and full darkness respectively. Seeds of *H. italicum* subsp. *siculum* are clearly negatively photosensitive, as darkness significantly improved germination response at all temperature regimes. Royal Botanic Gardens Kew (2021) indicates a 95% of germination at 20°C, 79% at 15°C and 77% at the alternating 25/15°C thermoperiod. Given the wide altitudinal range and the variety of habitats and substrata, it is likely that different populations may exhibit different germination behaviour as an adaptive response to distinct environmental factors. Further studies on a larger sample of populations will contribute to better define the germination requirements for this taxon.

79. *Helichrysum litoreum* Guss. (Asteraceae)

Accession data

Si: Sicily. Is. Eolie, Filicudi (Messina) (WGS84: 38.560834°N, 14.579723°E), coastal

cliffs, 60 m a.s.l., 01 Jul 2012, *P. Minissale* (UNICT_062, BGS-CT Catania Germplasm Bank).

Germination data

Pre-treatments: sterilization with a 1% sodium hypochlorite water solution for 3 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 5 ml of sterilized distilled water.

Sample size: 100 seeds for each test (25×4 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T ₁ [d]	T ₅₀ [d]	T _{max} [d]	MTG [d]
99.0%	constant 15°C	12/12h	9.0	12.4	27.0	14.3
94.0%	constant 20°C	12/12h	5.0	8.6	26.0	10.2
92.0%	constant 10°C	12/12h	5.0	9.0	27.0	10.6
81.0%	constant 20°C	0/24h	5.0	8.1	26.0	10.8
81.0%	constant 10°C	0/24h	12.0	12.1	30.0	14.0
80.0%	constant 15°C	0/24h	5.0	7.4	33.0	9.9

Observations

Helichrysum litoreum is an Italian Tyrrhenian endemic, occurring on sea cliffs, both on limestone and volcanic soils, and permanently affected by sea salt aerosol. The investigated population from Is. Eolie showed the highest germination response (> 90%) in the thermal range between 10°C and 20°C, with the maximum value (99%) at 15°C under 12/12h light conditions. The alternate exposure to 12 h photoperiod improved seed germination, while under full dark conditions the germination percentage was around 80%. A higher temperature (25°C) did not favour seed germination, which reached 74% with light exposure and only 47% with total darkness. Our results were significantly higher than those reported by Royal Botanic Gardens Kew (2021) at 15°C and 12/12 h (99% vs. 80%).

80. *Helichrysum nebrodense* Heldr. [= ? *H. nebrodense*] (Asteraceae)

Accession data

Si: Sicily. Madonie Mts., Polizzi Generosa (Palermo), Strada per Vallone Madonna degli Angeli (WGS84: 37.841902°N, 14.011112°E), 1047 m a.s.l., 21 Jul 2005, S. Pasta & L. Scuderi (BGS-CT/127LS/SP/05 Catania Germplasm Bank).

Germination data

Pre-treatments: sterilization with a 1% sodium hypochlorite water solution for 3 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 5 ml of sterilized distilled water.

Sample size: 100 seeds for each test (25×4 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T ₁ [d]	T ₅₀ [d]	T _{max} [d]	MTG [d]
80.0%	constant 15°C	0/24h	7.0	8.3	36.0	10.5

Observations

Helichrysum nebrodense is an orophilous Sicilian endemic species, confined to the mountain belt of Madonie Mts., on breccias and debris-flow deposits. The germination tests carried out at different constant temperatures under both light and dark conditions did not provide successful results, with a maximum of 80% of germinated seeds at 15°C and 0/24 h photoperiod. The worst response was at the highest temperature (25°C) with 47% and 19% of germination under light and full dark conditions respectively. Similarly, the lowest temperature (10°C) also gave a low germination rate, around 60%, both with and without light exposure. Overall, seeds of *H. nebrodense* do not seem to be significantly affected by light, except for higher temperatures when under 0/24 photoperiod germination dropped from 71% to 57% at 20°C and from 47% to 19% at 25°C. As far as we know from the literature, this is the first germination report for this taxon.

81. *Helichrysum panormitanum* Tineo ex Guss. subsp. *panormitanum* (Asteraceae)

Accession data

Si: Sicily. Castellammare del Golfo (Trapani), Mt. Inici (WGS84: 38.020834°N, 12.871389°E), 280 m a.s.l., 30 Jun 2013, S. Brullo (UNICT_087, BGS-CT Catania Germplasm Bank).

Germination data

Pre-treatments: sterilization with a 1% sodium hypochlorite water solution for 3 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 5 ml of sterilized distilled water.

Sample size: 100 seeds for each test (25×4 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T ₁ [d]	T ₅₀ [d]	T _{max} [d]	MTG [d]
90.0%	constant 15°C	12/12h	5.0	6.8	28.0	9.9
88.0%	constant 20°C	12/12h	5.0	7.6	33.0	11.2
87.0%	constant 10°C	12/12h	9.0	14.3	29.0	16.4
87.0%	constant 10°C	0/24h	13.0	14.9	33.0	18.1

Observations

Helichrysum panormitanum is a complex species endemic to NW Sicily and the Egadi islands, growing on sea cliffs not directly affected by sea splashes, which is characterized by a certain morphological variability among geographically distinct subtaxa (Iamomico & al. 2016). The typical form only occurs alongside the NW Sicilian coast, from Termini Imerese and Trapani (Brullo & Guarino 2018). Successful germination response was obtained ($\geq 87\%$) in the thermal range between 10°C and 20°C, with the highest percentage (90%) at 15°C and 12/12 h photoperiod. Seeds resulted as both thermal- and light-sensitive because tests carried out under full dark conditions provided lower germination percentages (but not at 10°C), while at 25°C the germination rate dropped down to 9% and 0% in light and total dark conditions, respectively. Our results differ from those of Royal Botanic Gardens Kew (2021), which reports (provenance unknown) 98% and 96% of germination at 20°C and 15°C, respectively, and 90% of germination with alternating temperature 25/10°C, all with a 12/12 h photoperiod. Further studies on a larger sample of populations and other experimental combinations will contribute to better define the germination requirements for this taxon.

82. *Helichrysum panormitanum* subsp. *stramineum* (Guss.) C. Brullo & Brullo (Asteraceae)

Accession data

Si: Sicily. Sferracavallo (Palermo), Capo Gallo (WGS84: 38.213056°N, 13.291667°E), rupi costiere, 280 m a.s.l., 31 May 2012, C. Brullo, S. Brullo, M. Patanè & D. Torrisi (UNICT_063, BGS-CT Catania Germplasm Bank).

Germination data

Pre-treatments: sterilization with a 1% sodium hypochlorite water solution for 3 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 5 ml of sterilized distilled water.

Sample size: 100 seeds for each test (25×4 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T ₁ [d]	T ₅₀ [d]	T _{max} [d]	MTG [d]
90.0%	constant 15°C	12/12h	6.0	6.6	30.0	8.7
81.0%	constant 15°C	0/24h	6.0	9.1	33.0	10.3
80.0%	constant 20°C	12/12h	4.0	5.9	22.0	8.1

Observations

According to Brullo & Guarino (2018), this taxon is confined to Capo Gallo, just north-west of Palermo. Seeds showed the best germination percentage (90%) at 15°C and 12/12

h photoperiod. Lower results, around 80%, were obtained at 15°C under full dark conditions and at 20°C with light exposure. The other tested temperatures provided further lower germination percentages, which ranged between 76% and 65% at 10°C under light and full dark conditions, respectively, while 63% was reached at 20°C and 0/24 photoperiod. The highest temperature (25°C) negatively affected the germination response (59% with 12/12 h photoperiod), as the absence of light also seems to do, given that seed germination was significantly lower under dark conditions, with the worst value at 25°C (17%). Our data disagree with those from Royal Botanic Gardens Kew (2021), giving on a sample of 19 seeds sown a 100% of germination at 15°C and 12/12 h.

83. *Helichrysum panormitanum* subsp. *messeriae* (Pignatti) C. Brullo & Brullo [*H. panormitanum*] (Asteraceae)

Accession data

Si: Sicily. Is. Egadi, Marettimo (Trapani), Punta Libeccio (WGS84: 37.959277°N, 12.046662°E), 17 m a.s.l., 21 Oct 2004, S. Pasta & L. Scuderi (BGS-CT 056GR5/04, Catania Germplasm Bank).

Germination data

Pre-treatments: sterilization with a 1% sodium hypochlorite water solution for 3 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 5 ml of sterilized distilled water.

Sample size: 100 seeds for each test (25 × 4 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T ₁ [d]	T ₅₀ [d]	T _{max} [d]	MTG [d]
99.0%	constant 15°C	12/12h	7.0	9.8	29.0	11.8
94.0%	constant 20°C	12/12h	4.0	11.4	30.0	13.2
93.0%	constant 15°C	0/24h	7.0	11.2	30.0	14.0

Observations

Due to the little availability of seeds and in accordance with the best germination results from allied taxa, the germination tests for this taxon, geographically limited to Marettimo (Egadi Is.), were performed at 15 and 20°C, under both 12/12h and 0/24h photoperiod, all giving successful germination results ($\geq 93\%$), with the maximum rate (99%) at 15°C and light exposure. Seed germination seems to be somehow improved by light exposure, because the germination percentages were lower under dark conditions, with major divergence at 20°C (69% vs. 94%).

**84. *Helichrysum preslianum* C. Brullo & Brullo subsp. *compactum* (Guss.) Maggio & al.
[*H. stoechas* subsp. *barrelieri* (Ten.) Nyman] (Asteraceae)**

Accession data

Si: Sicily. Mazara del Vallo (Trapani), Torretta Granitola (WGS84: 37.599977°N, 12.631203°E), 1 m a.s.l., 13 Jun 2005, S. Pasta & L. Scuderi (BGS-CT 101LS/SP/05, Catania Germplasm Bank).

Germination data

Pre-treatments: sterilization with a 1% sodium hypochlorite water solution for 3 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 5 ml of sterilized distilled water.

Sample size: 100 seeds for each test (25 × 4 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T ₁ [d]	T _{s0} [d]	T _{max} [d]	MTG [d]
81.0%	constant 15°C	12/12h	6.0	7.4	29.0	9.6

Observations

The population occurring on calcarenite outcrops alongside the sea cost of Mazara del Vallo, namely in the area of Torretta Granitola, were originally described as *H. caespitosum* var. *compactum* Guss. and recently revalued by Maggio & al. (2016) as *H. preslianum* C. Brullo & Brullo subsp. *compactum* (Guss.) Maggio, Bruno, Guarino, Senatore & Ilardi, also based on secondary metabolite patterns. The germination tests carried out on this population provided satisfactory results (81%) only at 15°C and 12/12h photoperiod. Conversely, at the other tested temperatures, seed germination ranged between 74% and 79% at 15 and 25°C, respectively, with light conditions, and between 72% (15°C) and 75% (20 and 25°C) under total darkness. The worst germination response was obtained at 10°C, with only 14% of germinated seed under light exposure and 59% with 0/24 photoperiod. Light just little improved the germination rate, except for the lowest temperature which seems to have benefited by total darkness.

85. *Helichrysum stoechas* (L.) Moench [*H. stoechas* subsp. *stoechas*] (Asteraceae)

Accession data

Si: Sicily. Gela (Caltanissetta), Passo delle Pantanelle (WGS84: 37.047810°N, 14.386846°E), xeric steppe grasslands on limestone, 120 m a.s.l., 20 Jun 2005, S. Sciandrello (BGS-CT SS107/05, Catania Germplasm Bank).

Germination data

Pre-treatments: sterilization with a 1% sodium hypochlorite water solution for 3 minutes followed by 3 rinses in sterile distilled water.

Germination medium: 3 sheets of sterilized filter paper (Whatmann No. 1), imbibed with 5 ml of sterilized distilled water.

Sample size: 100 seeds for each test (25×4 replicates).

Germination	Thermoperiod	Photoperiod [light/dark]	T ₁ [d]	T ₅₀ [d]	T _{max} [d]	MTG [d]
82.0%	constant 15°C	0/24h	6.0	5.6	29.0	7.8
81.0%	constant 15°C	12/12h	6.0	7.4	29.0	9.6

Observations

This is the most widespread taxon among the Mediterranean group, which in Italy is native along the Tyrrhenian coasts of Liguria, Tuscany and Latium, and in the south-eastern coast of Sicily, where it can be found in the retrodunal areas, fixed dunes, and coastal cliffs, between 0 and 1000 m. The studied Sicilian population showed good germination percentages (> 80%) at 15°C, both under 12/12 h and 0/24 h photoperiod. The other tested temperatures provided lower germination percentages, ranging between 65% (20°C, 12/12h) and 76% (10°C, 0/24h). No significant difference was detected between the different applied photoperiods, but little lower germination percentages were obtained at 10° C (56% vs. 76%) and 20°C (65% vs. 74%) under light exposure. Our results are in accordance with Royal Botanic Gardens Kew (2021), giving a similar germination percentage (83%) at 15°C and 12/12h condition (unknown provenance).

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