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Apium graveolens PI 181714 is a source of resistance to *Fusarium oxysporum* f. sp. *apii* race 4 in celery (*A. graveolens* var. *dulce*)

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Abstract

Celery has little genetic diversity and is highly susceptible to the new fungal pathogen *Fusarium oxysporum* f. sp. *apii* (*Foa*) race 4. After screening an *Apium graveolens* germplasm collection for resistance to *Foa* race 4, we crossed celery cv. 'Challenger', which is *Foa* race 2-resistant but *Foa* race 4-susceptible and *A. graveolens* PI 181714, which is *Foa* races 2- and 4-resistant but non-celery type. After selfing F_1s, we screened the F_1S_1 for race 4-resistance and celery-type and then selfed selected F_1S_1. Greenhouse and field trials indicate that three selected F_1S_2 families (76–8-4, 76–8-27 and 76–8-36) are suitable as germplasm for celery breeders for resistance to *Foa* race 4. A F1S3 76–8–36-124 is either fixed or nearly so for resistance to *Foa* races 4 and 2. Furthermore, quantitative PCR indicates that PI 181714 is resistant, rather than tolerant, to *Foa* races 4 and 2, and that this resistance has been introgressed into F1S3 76–8–36-124.

KEYWORDS
Apium graveolens, breeding, celery, disease resistance, *Fusarium oxysporum* f. sp. *apii*, resistance breeding

1 | INTRODUCTION

The progenitor of celery, *A. graveolens* L. var. *graveolens*, was domesticated into three crops: celery (*var. dulce*); celeriac (synonym, celery root) (var. *rapaceum*) and cutting celery (synonyms, Chinese local celery, leaf celery and smallage) (var. *secalinum*) (Quiros, 1993). All varieties are sexually compatible. As with many crops (Purugganan, 2019; van de Wouw et al., 2010), celery has little genetic diversity (Quiros, 1993), and genes for disease resistance have to be introgressed from other *A. graveolens* varieties (Quiros, 1993). For example, after introgression of gene(s) for resistance from celeriac (Orton, Durgan, & Hulbert, 1984; Orton, Hulbert, et al., 1984), *Fusarium oxysporum* f. sp. *apii* (*Foa*) race 2 (*Foa*R2) has been adequately controlled by resistant cultivars, for example, cv. 'Challenger' (Daugovish et al., 2008; Subbarao & Elmer, 2002).

California is the major producer of celery in the United States, with production of 744 000 tonnes in 2018 (USDA, 2020). *Foa* race 4 (*Foa*R4), a new fungal pathogen of celery, was first reported in Camarillo, California, USA in 2017 (Epstein et al., 2017; Epstein et al., 2022); it is comparatively unrelated to *Foa*R2 (Henry et al., 2020). FoaR4 causes Fusarium wilt of celery, which can cause complete crop loss, particularly when temperatures are 22°C and above (Kaur et al., 2022). Because resistance is the best strategy for controlling crop diseases caused by *F. oxysporum* (Chitwood-Brown et al., 2021), we screened 243 accessions for resistance, selected an *A. graveolens* accession (PI 181714) that is resistant to *Foa*R4 and *Foa*R2, and crossed it with *Foa*R2-resistant (but *Foa*R4-susceptible) celery cv. 'Challenger'. We then selected for *Foa*R4-resistance in lines with celery-type in three subsequent selfed generations (the F_1S_1, F_1S_2 and F_1S_3). Here, we show a combination of greenhouse, field and laboratory data that indicate that (1) PI 181714 is a source of resistance to *Foa*R4 for celery, (2) PI 181714 is immune to *Foa*R4 and (3) selected F1S2 and F1S3 will be useful for breeding celery cultivars.

2 | MATERIALS AND METHODS

2.1 | Plants and *Foa*R4 and *Foa*R2

University of California (UC), Davis, has a celery germplasm collection that contains materials from the USDA (https://www.ars.usda.gov/northeast-area/geneva-ny/plant-genetic-resources-unit-pgru/docs/celery-collection/), and additional materials obtained by UC researchers (Supporting information, Table S1). Air-dried seeds were stored at 10.5°C and 30% humidity. Additional celery cultivars were obtained from commercial sources: 'Challenger', 'Conquistador' and 'Sonora' (Syngenta, Greensboro, NC), 'CG390' (Cal Grow, Santa Maria, CA), 'Tall Utah 5270R Improved' (Ferry Morse, Norton MA) and 'Earthrace' (Rijk Zwaan, provided by White Seed, Oxnard, CA).

*Foa*R4 and *Foa*R2 are clonal populations that are invariant in California within race (Epstein et al., 2017). For greenhouse trials, we used California isolates *Foa*R4274.AC and *Foa*R2 207.A, from Camarillo and Santa Maria, California, respectively; cultures are available from the USDA-ARS NRRL (https://nrrl.ncaur.usda.gov/), and genome assemblies are in GenBank (https;//www.ncbi.nlm.nih.gov/) as JAAOOQ000000000 and JAAOOO000000000, respectively (Henry et al., 2020).

2.2 | Greenhouse assay for screening the germplasm collection and the progeny

The greenhouse assay was described previously (Kaur et al., 2022). Briefly, each 2-month-old seedling was transplanted as a plug plant into a tube with either uninfested soil or soil infested with either *Foa*R4 or when indicated, *Foa*R2. For screening the germplasm collection for resistance to *Foa*R4, every trial included 'Challenger' as a susceptible control treatment. Plants were incubated in a completely randomized design and incubated at 27–29°C. At harvest, the washed roots and crowns were scored as follows: 0, asymptomatic; 1, characteristic discoloration in the fine roots but none elsewhere; 2, characteristic discoloration in the main root but none in the crown; 3, discoloration in the crown vasculature but on < 1/4 of the circumference of the vascular ring; 4, discoloration in the crown vasculature on >1/4 of the circumference of the vascular ring; and 5, plant dead.

2.3 | Selective breeding

The most promising accessions from the germplasm collection were rescreened in the greenhouse in infested soil and then, depending on results, tested in a field trial and crossed with 'Challenger'. Crosses designated as '76-' were from a single 'Challenger' X a pool of pollen from three PI 181714 plants. Twenty-five random F_1s were selfed (F_1S_1) and assayed for *Foa*R4-resistance in the greenhouse; seven of the most resistant families were included in field trials in *Foa*R4-infested soil. In a *Foa*R4-infested field with approx. 800 F_1S_1 76–8, plants were cut at 10 cm above the soil line, and 36 plants with the best celery-type were selected. The selected 76–8 plants were dug, washed and examined for symptoms; asymptomatic plants were used to produce rooted cuttings. After the rooted cuttings were assayed for *Foa*R4-resistance, 19 of the selected 76–8 mothers were selfed. After assaying the F_1S_2 for *Foa*R4-resistance and celery-type in the greenhouse, testing was performed in replicated field plots as indicated below. In addition, nine F_1S_2 76–8-36 were selfed, and a F_1S_3 was screened in the greenhouse for *Foa*R4 and *Foa*R2-resistance.

2.4 | Field trials

Trials were performed in fields that were naturally infested with either *Foa*R4 or *Foa*R2 but not both; races were identified as described previously (Epstein et al., 2017; Henry et al., 2020). Plots were managed in accordance with standard commercial practice for celery (Daugovish et al., 2008). Two-month-old plug plants were transplanted into 102 cm-wide beds; each bed had two rows, with plants in a row at 18–19 cm spacing. Plots were 7.6 m long. At harvest, selected plants were dug, washed and evaluated for symptoms of *Foa* infection and for celery-type.

For the featured *Foa*R4 trial in Camarillo CA, the two parents and seven F_1S_2 were planted into a completely randomized design with four replicate plots/accession. Plants were transplanted on 18 August 2021. After seven weeks, 20 plants/plot were randomly selected and flagged. At harvest-time on 16 November, the previously selected plants were evaluated.

For the featured *Foa*R2 trial in Santa Maria, CA, 20 randomly selected plants from each of three replicates in a randomized complete block design were evaluated at harvest-time on 16 September 2021.

2.5 | Quantitative PCR (qPCR) of *Foa*R4 and *Foa*R2 in crowns

Infestation of greenhouse soil was done as described previously (Kaur et al., 2022). There was a three-factorial design with three genotypes (two parents and F_1S_3 76–8–36-124), three infestations (uninfested and infested with either *Foa*R4 or *Foa*R2) and two sampling times (10- and 20-days post-transplantation, dpt); plants were arranged in a completely randomized design with five replicates. Harvesting of crowns and qPCR was performed as described previously (Kaur et al., 2022) except that the lyophilized crowns were pulverized by grinding in liquid N_2 with a mortar and pestle. Briefly, DNA was extracted from pulverized crown tissue with a Zymo Research Quick-DNA Fecal/Soil Microbe Miniprep Kit (Irvine, CA). Total DNA from the celery crowns was determined with a dsDNA BR Assay Kit (Invitrogen/Life Technologies, Carlsbad, CA) in a Qubit 2.0 fluorometer (Invitrogen/Life Technologies). For each sample, there were two reactions with 10 ng of total DNA. We used FoaR4-MC2 (107 bp amplicon) and FoaR2-MC1 (153 bp amplicon) race-specific primers and PrimeTime 5′ nuclease probes (Kaur et al., 2022); probes have a 5′ 6-FAM reporter dye, an internal ZEN quencher and a 3' Iowa Black quencher (IDT, Coralville, IA). PCR conditions were 3 min at 95°C and 40 cycles of 15 s at 95°C, 30 s at 56°C and 60 s at 72°C. All runs

included no-template controls and a serial dilution of DNA from a pure culture of the appropriate *Foa* race, which was used as an external standard for *Foa* quantification. After the quantity of *Foa* DNA was interpolated from the standard, we normalized the femtograms *Foa* DNA per nanogram celery DNA.

2.6 | Statistical analyses

For the data shown in Figure 2, *Foa* concentrations in crown tissue of plants grown in infested soil were log-transformed in order to meet the assumption of homogeneity of variance. Log *Foa* concentration data were analysed by ANOVA. For the data shown in Table 1, for a comparison of genotypes in a field, the fraction of plants per replicate of selected binary phenotypes were compared by the non-parametric Dunn method for joint ranking. For the data shown in Table 2, for a comparison of genotypes in a completely randomized greenhouse trial, counts in 2 × 2 contingency tables were compared by chi-square likelihood ratios. When indicated, goodness of fit tests were used to examine indicated segregation ratios. All data were analysed with JMP Pro 16 (SAS Institute, Cary NC, USA).

3 | RESULTS

3.1 | Screening of germplasm for resistance to *Foa*R4

We screened 243 accessions for resistance to *Foa*R4: 125 celery; 68 celeriac; 31 cutting celery; six celery X celeriac; five other *A. graveolens* varieties and eight miscellaneous *Apium* spp. (Supporting information, Table S1). We selected *A. graveolens* USDA PI 181714, which was collected from Turkey, for potential resistance against *Foa*R4.

3.2 | Parental phenotypes

Images of the parents, celery cv. 'Challenger' and PI 181714, are shown in Figure 1. 'Challenger' has the standard celery-type phenotype; the edible 'stalks' are erect, wide petioles that are solid in cross-section. In contrast, the non-celery type PI 181714 has a semi-prostrate habit (Figure 1a left side), with comparatively narrow diameter petioles that have air cavities. PI 181714 has a very vigorous root system (Figure 1b left side) and resistance to *Foa*R4 (Figure 1c, second from left) and to *Foa*R2. 'Challenger' is also *Foa*R2-resistant but *Foa*R4-susceptible (Figure 1c right side). In 'Challenger', *Foa*R4 causes a brown discoloration in the vasculature in multiple subterranean tissues: crown (Figure 1d arrows), transition zone between the crown and roots (Figure 1e) and roots (Figure 1f). Plants with an extensive vascular discoloration that affects more than one fourth of the crown circumference are often stunted (Figure 1g). A cross-section of a PI 181714 crown, without any vascular discoloration, is shown in Figure 1h. Non-celery features of PI 181714 include 'non-solid' petioles, that is, with airspaces within the tissue (Figure 1i), a less compact arrangement of the petioles and more numerous petioles that are of smaller diameter (Figure 1i–j) than in 'Challenger' (Figure 1k–l).

3.3 | Biomass of *Foa*R4 and *Foa*R2 in the parents

Foa concentration in crown tissue, as measured by qPCR, provides a measure of celery susceptibility (Kaur et al., 2022). Quality control procedures indicated that qPCR efficiencies for *Foa*R4 and *Foa*R2

TABLE 1 Evaluation of *Foa* race 4-resistance and celery-phenotype of the two parents of a F1 and seven F1S2 in a field trial in soil that was infested with *Foa* race 4†

		Fraction of survivors‡				
(Parent of F1) or F1S2 family that was derived from F1S1 76–8	Fraction that died	Asymptomatic above-ground	Asymptomatic below-ground§	Solid petioles	Celery-type growth habit¶	Compactly-arranged petioles
(PI 181714)	.00 a	1.00 a	1.00 a	.00 b	.00 c	.00 b
(Challenger)	.28 b	.49 b	.15 b	1.00 a	1.00 a	1.00 a
F1S2 76–8-4	.00 a	1.00 a	.90 ab	1.00 a	.96 abc	.96 ab
F1S2 76–8-15	.00 a	1.00 a	.92 ab	1.00 a	.99 ab	.99 ab
F1S2 76–8-19	.00 a	.98 ab	.86 ab	1.00 a	.86 abc	.86 ab
F1S2 76–8-27	.00 a	1.00 a	.85 ab	1.00 a	.85 abc	.85 ab
F1S2 76–8-28	.01 ab	.95 ab	.83 ab	1.00 a	.78 abc	.78 ab
F1S2 76–8-36	.00 a	1.00 a	.98 a	1.00 a	.67 abc	.67 ab
F1S2 76–8-110	.00 a	1.00 a	.94 ab	1.00 a	.14 b	.14 ab

†There were four replicate plots/family or accession in a completely randomized design. Twenty plants/plot were randomly selected and evaluated. Within a column, means followed by the same letter were not significantly different by the non-parametric Dunn method for joint ranking ($\alpha = .05$), which uses the Bonferroni adjustment for multiple comparisons.

‡Of the randomly selected plants, mortality was limited to 28% of the Challenger and a single plant in the F1S2 76–8-28.

§Vascular discoloration-based score of 0, with none of the diagnostic discoloration in the main roots or crown.

¶Celery-type has fewer and wider petioles than non-celery-type.

TABLE 2 The parents, F1S1 76–8, three F1S2 and F1S3 76–8–36-124: The percentage with symptoms of Fusarium wilt in *Foa* race 4-infested soil and celery versus non-celery-type in a greenhouse trial†

		Symptoms of Fusarium wilt from *Foa* race 4							Plant architecture§				
		Vascular discoloration-based score from 0 (asymptomatic) to 5 (dead)‡								Growth habit type			
		0	1	2	3	4	5	Asymptomatic above-ground	All solid petioles	Celery	Mix of celery and non-celery	Non-celery	
Generation	Plant ID	Plants, %											n¶
F1 parent	PI 181714	**94**	0	0	4	2	0	**100**	0	0	0	100	47
F1 parent	cv. Challenger	0	0	0	0	38	63	0	**100**	**100**	0	0	48
F1S1	76–8	54	3	1	15	21	6	64	19	53	44	3	80
F1S2	76–8-4	49	11	0	11	16	11	73	100+	91	9	0	79
F1S2	76–8-27	68	0	2	12	18	0	82	100+	84	16	0	125
F1S2	76–8-36	84	13	4	0	0	0	100+	100+	63	37	0	80
F1S3	76–8–36-124	95+	0	0	5	0	0	99+	100+	85	15	0	80

†Two-month-old plugs were transplanted into a completely randomized design. There were three pathogen treatments: *Foa* race 4-infested soil (*n* shown in last column); *Foa* race 2-infested soil ($n = 20$), with symptomatic scores shown in Supporting information, Table S5; and uninfested soil ($n = 10$), which were all asymptomatic. Values of parental phenotypes that are desired in the progeny are bolded.
‡0, asymptomatic; 1, characteristic discoloration in the fine roots but none elsewhere; 2, characteristic discoloration in the main root but none in the crown; 3, discoloration in the crown but on <1/4 of the vasculature; 4, discoloration in the crown on >1/4 of the vascular ring; and 5, plant dead.
§Data on plant architecture were based on the survivors from the *Foa*-race 4 treatment (because dead plants cannot be scored for these phenotypes) and the additional 30 plants. Celery has fewer and wider petioles than non-celery-type.
¶The number of plants (=replicates) in *Foa* race 4-infested soil.
+A chi-square likelihood ratio analysis of 2 × 2 contingency tables was performed only on data in the four columns with a bolded parental phenotype. Values marked with a + are indistinguishable ($p > .05$) from those of the parent with the desired phenotype.
Computed *p*-values are shown in Supporting information, Table S6.

were ≥95%, and there was no amplification of either *Foa*R4 or *Foa*R2 in any of the uninfested controls. In an ANOVA of the log concentration of *Foa*R4 in crowns from plants in *Foa*R4-infested soil, genotype*dpt ($p < .001$), genotype ($p = .0005$) and dpt ($p < .0001$) were highly significant ($n = 5$) (Supporting information, Table S2). At 10 dpt, except for one 'Challenger' that had symptoms on its fine roots, none of the plants in infested soil were symptomatic. At 20 dpt, three of the 'Challenger' were in early stages of vascular discoloration (score = 2 or 3). Based on contrast analysis, at 20 dpt, PI 181714 had significantly less ($p < .001$) *Foa*R4 (Figure 2a) in crowns than 'Challenger'. Consequently, PI 181714 is at least partly immune, rather than tolerant, to *Foa*R4. In an ANOVA of the log concentration of *Foa*R2 in crowns of plants in *Foa*R2-infested soil (Figure 2b), genotype*dpt ($p = .23$), genotype ($p = .11$) and dpt ($p = .53$) were non-significant (Supporting information, Table S2). Previously, we showed that the *Foa* race 2-resistant 'Challenger' had a significantly lower concentration of *Foa* race 2 in their crowns than a race 2-susceptible celery (Kaur et al., 2022). Consequently, if *Foa* biomass in crowns is a marker for resistance, PI 181714 has an indistinguishable level of *Foa*R2-resistance as 'Challenger'.

3.4 | Breeding and selection process

Progeny were derived from 'Challenger' (celery^{+} *Foa*R2^{R} *Foa*R4^{S}) X *A. graveolens* PI 181714 (celery^{-}*Foa*R2^{R} *Foa*R4^{R}). The selection process is summarized in the materials and methods section, Supporting information Figure S1 and below.

3.5 | *Foa*-resistance and celery-type phenotypes of F1S2 and F1S3

3.5.1 | Preliminary greenhouse assays for *Foa*R4-resistance and celery phenotypes

Based on greenhouse and preliminary field trials (data not shown), we selected the F_1S_1 76–8 and produced 20 F_1S_2 (Supporting information, Table S3). Of these F_1S_2, we selected six with the most potential for *Foa*R4-resistance and celery-type and one (76–8-110) with excellent *Foa*R4-resistance but more non-celery-type.

3.5.2 | F1S2 in a *Foa*R4-infested field

The seven selected F_1S_2 and their parents were planted in a field trial in soil that was naturally infested with *Foa*R4 (Table 1). There were significant ($\alpha = .05$) differences between the two parents. None of the PI 181714 died, and none had tissue with either above-ground or below-ground symptoms of *Foa*R4-infection. However, none of the PI 181714, in contrast to 100% of the 'Challenger', had a celery-phenotype: petioles that were solid in cross-section and erect petioles in a

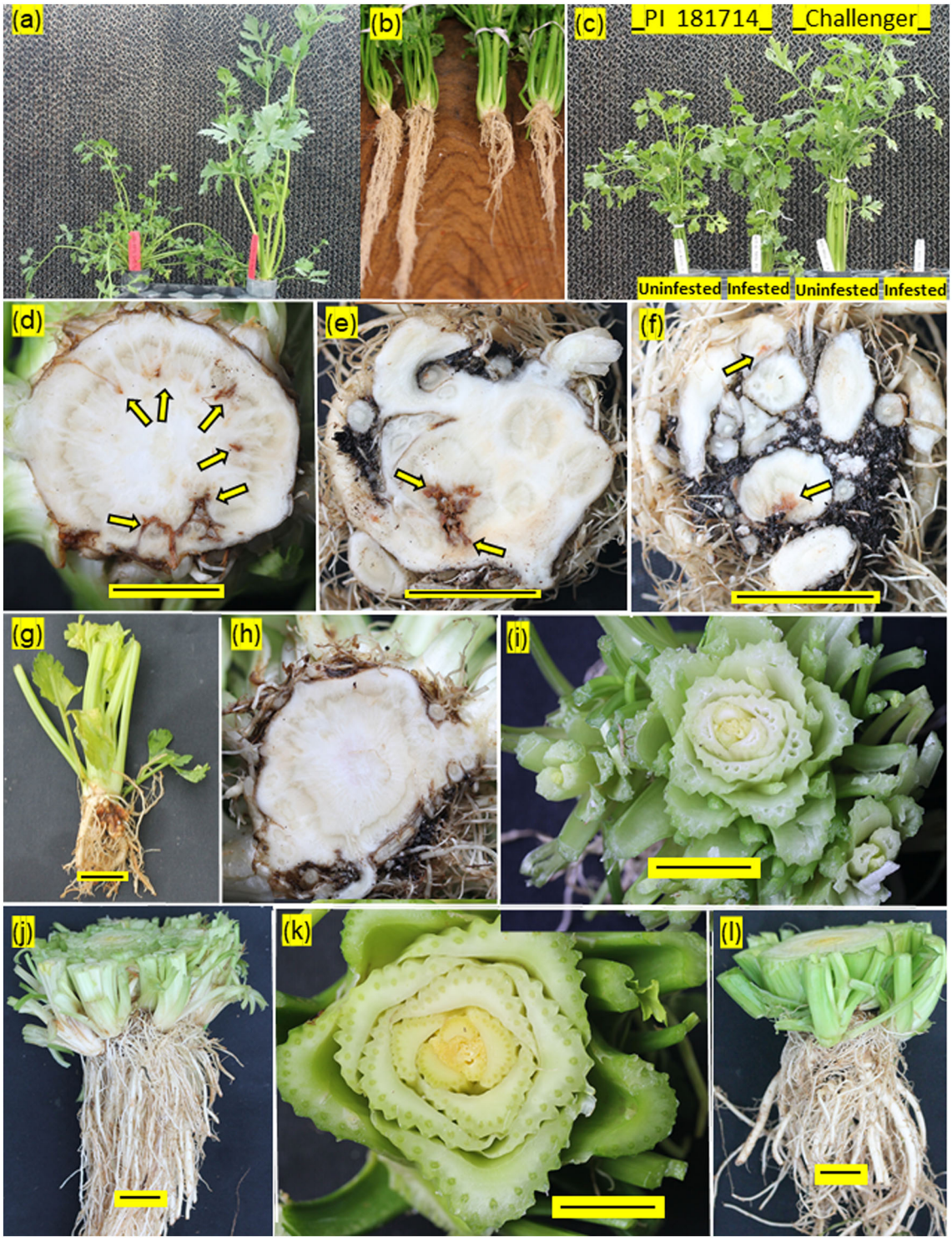

FIGURE 1 The two *Apium graveolens* parents (celery cv. Challenger and PI 181714) were grown in either soil infested with *Fusarium oxysporum* f. sp. *apii* race 4 (*Foa*R4) or in uninfested soil (a-b) or where indicated (c). (a–c) Plants from a greenhouse trial 6 weeks after transplantation. (a) Left, PI 181714 petioles are non-erect, non-celery type. Right, Challenger petioles are erect. (b) Left, PI 181714 produces more roots than Challenger, right. (c) PI 181714 is resistant to *Foa*R4, whereas Challenger is highly susceptible. (PI 181714 only appears to be erect because we staked plants in the greenhouse). (d–l) Plants at harvest-time from a 2021 field trial in soil infested with *Foa* race 4. (d–f) Cross-sections of Challenger with classic vascular discoloration (vd) symptoms indicated by arrows. (d) A crown; this plant had a vd score of '3'. (e) The transition zone between the main roots and the crown. (f) The main roots. (g) Major stunting in Challenger; this plant had a vd score of '4'. (h,i,j) PI 181714 (h) cross-section of a crown, without any vascular discoloration. (i) Top view of a cross-section of PI 181714 petioles, which are not solid, smaller diameter and in a less compact arrangement than in a celery-type. (j) A side view of the previous image. (k–l) An asymptomatic Challenger, which presumably escaped infection in the field. (k) Cross-section of the petioles. (l) A side view of the previous. Size bars = 2 cm

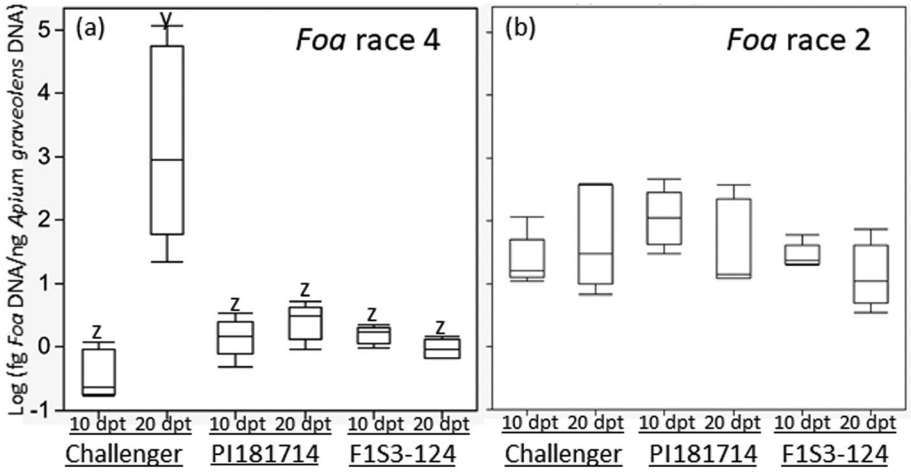

FIGURE 2 Box plots of the log concentration of *F. oxysporum* f. sp. *apii* (a) race 4 (*Foa*R4) and (b) race 2 (*Foa*R2) in *Apium graveolens* crowns 10- and 20-days post-transplantation (dpt) into either uninfested soil (data not shown) or soil that was infested with either *Foa*R4 or *Foa*R2 ($n = 5$). Plants were either the parentals cv. Challenger and *A. graveolens* PI 181714 or their F1S3 76–8–36-124 (F1S3–124). No *Foa*R4 or *Foa*R2 DNA was detected in any of the crowns of plants in uninfested treatments (data not shown). For Figure 2a, means followed by the same letter were not significantly different by Tukey's HSD at $\alpha = .05$. The 10 and 20 dpt time points were preselected to represent presymptomatic and early-symptomatic times, respectively, for susceptible genotypes. Phenotypes of plants in a concurrent greenhouse trial 35 dpt are shown in Table 2 for *Foa*R4-infested soil and in Supporting information Table S5 for *Foa*R2-infested soil.

compact arrangement. Although all 7 F_1S_2 had solid petioles, none of the F_1S_2 has 100% celery architecture. A representative of the three selected F_1S_2 (76–8-4 and 76–8-27 and 76–8-36) is shown in Figure 3; a representative of the celery-type of the second-tier F_1S_2 is shown in Supporting information, Figure S2.

3.5.3 | A limited trial of F1S2 in a *Foa*R2-infested field

Two of the three selected F_1S_2 (76–8-4 and 76–8-36) were also tested in a *Foa*R2-infested field, along with two other F_1S_2 (76–8-15 and 76–8-10) and three control cultivars (Supporting information, Figure S3). 76–8-4 and 76–8-36 were first-tier selections (Figure 4); 76–8-15 lacked the vigour of a first-tier selection, and 76–8-10 had insufficient celery-type.

3.5.4 | F_1S_3 76–8–36-124 (F_1S_3–124) appears to be homozygous for resistance to *Foa*R4

Nine F_1S_2 76–8-36 were selfed and then evaluated for *Foa*R4-resistance and celery-type (Supporting information, Table S4). Using a classification system for resistance with 0 to 2 as resistant, the nine F_1S_3 had a relatively high percentage (≥86%) of *Foa*R4-resistant progeny; 98% of the F_1S_3–124 plants were classified as resistant, as were those of the resistant parent PI 181714, compared to 0% of the susceptible parent cv. 'Challenger'. In contrast, celery-type is highly variable in the nine F_1S_3 with 82% celery-type in the F_1S_3–124 amidst a range of celery-type ranging from 25% to 93%.

3.5.5 | Further characterization of the parents, the F_1S_1, three F_1S_2 and a F_1S_3 in *Foa*R4- and *Foa*R2-infested soil in the greenhouse

As expected, 'Challenger' is susceptible to *Foa*R4, and PI 181714 is resistant to *Foa*R4 but non-celery type (Table 2). Both parents and all progeny are resistant to *Foa*R2 (Supporting information, Table S5). Based on an assessment of both below-ground tissue for the characteristic vascular discoloration and the above-ground tissue for either characteristic chlorosis, wilting, stunting or death, F_1S_3–124 and PI 181714 are indistinguishable for resistance to *Foa*R4 ($P > .05$) (Supporting information, Table S6). Consequently, based on symptoms, F_1S_3–124 has acquired PI 181714's requisite resistance genes to *Foa*R4 and is either fixed, that is, homozygous, for resistance or is as fixed as PI 181714. The solid petiole phenotype is fixed in the three F_1S_2. All of the progeny are still segregating for a celery-type habit.

3.5.6 | Genes introgressed from PI 181714 confer resistance/immunity to *Foa*R4 in the F1S3 breeding line

At 20 dpt, the log concentration of *Foa*R4 in F_1S_3–124 and PI 181714 *in planta* were indistinguishable statistically ($p = .32$, contrast analysis); both lines had significantly less *Foa*R4 than in 'Challenger' (Figure 2a). In addition, F_1S_3–124 at both times points (Figure 2b) has no significantly greater concentration of *Foa*R2 than either parent ($p = .18$, F test). Consequently, based on qPCR, F_1S_3–124 apparently has inherited the requisite resistance to *Foa*R4 and *Foa*R2 from its parents.

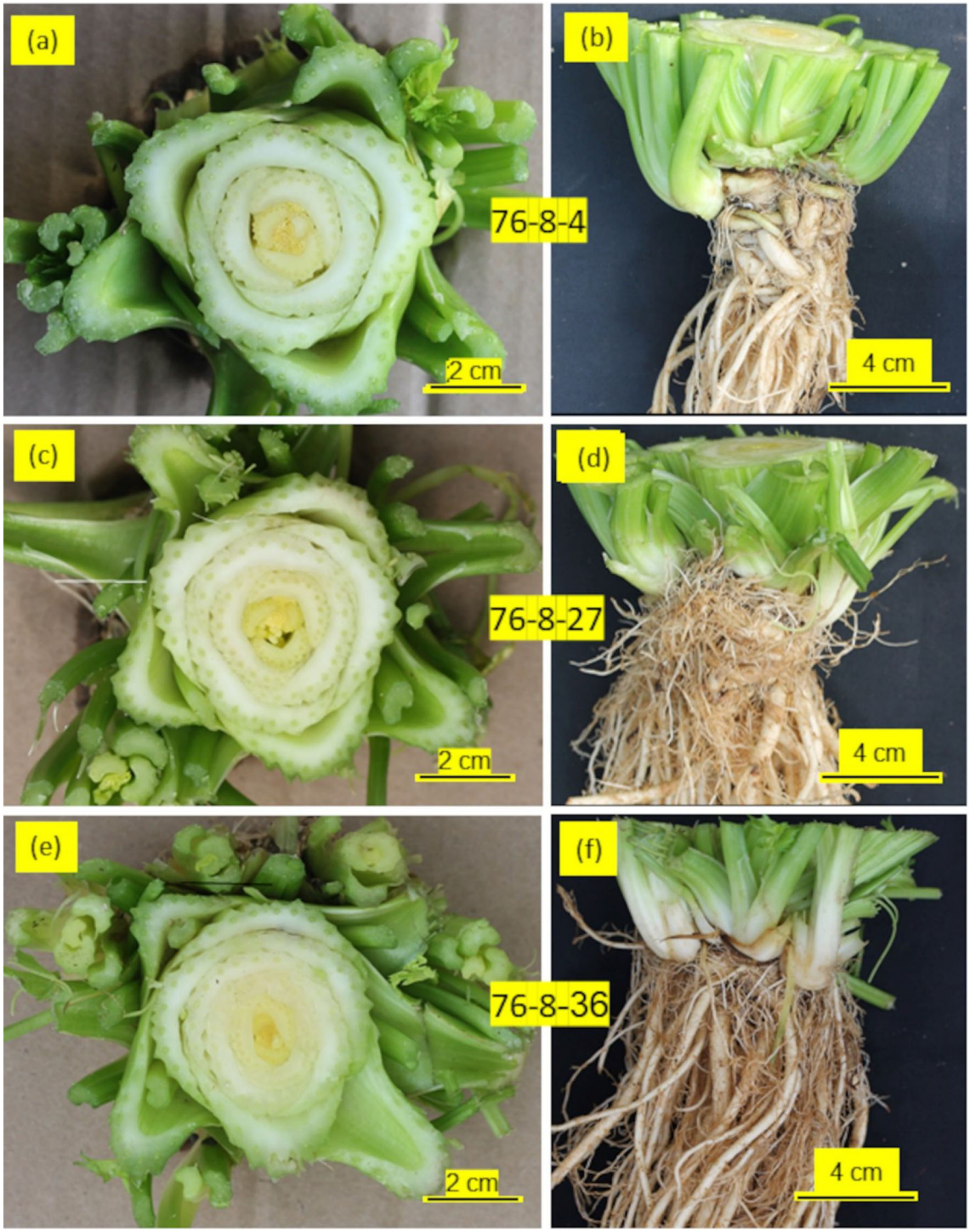

FIGURE 3 Field-grown F1S2 from a trial in *Foa* race 4-infested soil in Camarillo, CA in 2021. Left, a cross-section, and right, a longitudinal view of representative celery plants from the three selected F1S2 families (76–8-4, 76–8-27 and 76–8-36)

4 | DISCUSSION

Here, we demonstrate that PI 181714 is resistant to *Foa*R4 and that three selected F_1S_2 and a F_1S_3 from *A. graveolens* var. *dulce* 'Challenger' X *A. graveolens* PI 181714 carry the resistance to *Foa*R4. The F_1S_3 is apparently fixed for resistance. In addition, both parents and the F_1S_2 and F_1S_3 are resistant to *Foa*R2. Although the new germplasm will be useful for celery breeding, breeders should be aware of three issues. (1) Selection will require screening against the pathogen. In our view, in order to avoid declaring escapes as falsely resistant, breeders should use greenhouse assays with a consistent inoculum and a conducive temperature (Kaur et al., 2022). (2) Because *Foa*R4 is so aggressive and it is unclear how the pathogen can be removed once it is introduced into soil, it seems prudent to limit

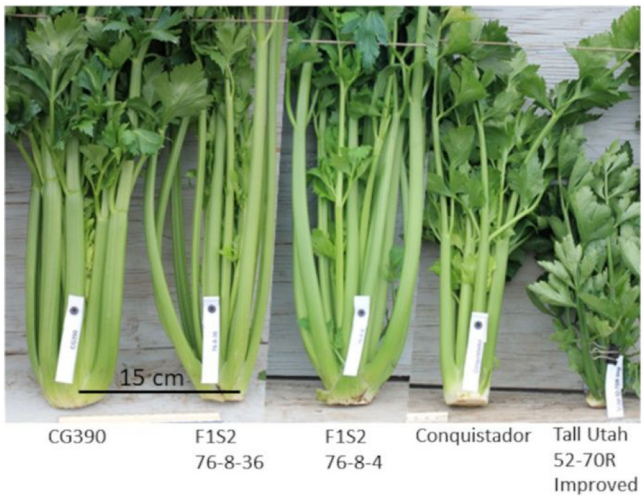

FIGURE 4 Field-grown plants from a trial in *Foa* race 2-infested soil in Santa Maria, CA in 2021. From left to right: CG390, a Challenger-like cultivar; F1S2 76–8-36; F1S2 76–8-4; Conquistador, which is a popular contemporary celery but is moderately *Foa* race 2-susceptible; and Tall Utah 52-70R improved, which is an older cultivar that is no longer grown in California, partly because it is *Foa* race 2-susceptible

greenhouse trials to either secure facilities or to areas in which neither celery nor other *A. graveolens* are grown. (3) For the same reasons, field tests should only be done in areas in which the pathogen is already present.

This is the first report of *A. graveolens* germplasm that controls *Foa*R4. Here, using qPCR, we demonstrate that PI 181714 is, at least partly, immune rather than tolerant, to *Foa*R4 and is as resistant to *Foa*R2 as 'Challenger'; we previously showed that 'Challenger' is similarly at least partly immune to *Foa*R2 (Kaur et al., 2022). In an examination of the progenitor of 'Challenger', Orton et al. (Orton, Durgan, & Hulbert, 1984) concluded that one major and perhaps one minor gene from the celeriac parent conferred *Foa*R2-resistance. Introgression of more than one gene from PI 181714 also may be required for resistance to *Foa*R4. Based on the parental phenotypes shown in Table 2, particularly in the greenhouse assay, which has high disease pressure due to a high inoculum concentration and temperature, it seems reasonable to score a vascular discoloration (vd) score of either 4 or 5 as a susceptible genotype and 0 or 1 and probably 2 as a resistant genotype. However, we do not know how to interpret scores of vd = 3. If we score vd = 3 as indicative of a susceptible genotype, then F_1S_1 76–8 and F_1S_2 76–8-4 deviate significantly from a 3 resistant: 1 susceptible expected for a single dominant gene ($p = .0003$ and .003 with $\chi^2 = 13.1$ and 8.5, respectively, df = 1). F_1S_2 76–8-27 segregates 3 resistant: 1 susceptible, consistent with having one segregating resistance gene and one fixed resistance gene, regardless of whether vd = 3 is classified as a susceptible or a resistant phenotype ($p = .16$ and $p = .09$ with $\chi^2 = 1.9$ and 2.9, respectively, df = 1). Alternatively, if we score vd = 3 as indicative of a resistant genotype, then F_1S_1 76–8 and F_1S_2 76–8-4 are consistent with 3 resistant: 1 susceptible expected for a single dominant gene ($p = .61$ and .56 with $\chi^2 = .27$ and .34, respectively, df = 1). We note that in our assays for *Foa*R4--resistance, we have greatest confidence with assays on unwounded seedlings; all quantified results shown here utilized uninfected two-month-old seedlings that were transplanted as plugs into infested soil into either the greenhouse or the field. However, in our selection procedure for *Foa*R4-resistance, we also used an assay with clones/rooted vegetative cuttings of plants that were retrieved from a field trial because we wanted to compensate for two mitigating circumstances: (1) the greenhouse is a comparatively poor location for screening for celery-type; but (2) our field trials did not have as much disease pressure as in our greenhouse trials, and we wanted to limit the number of plants that were healthy only because they had escaped infection rather than being *Foa*R4-resistant.

Many celeriac accessions in the USDA and UC *Apium* germplasm collections appear to have genes that control *Foa*R2 (Kaur and Epstein, unpublished), which is consistent with the successful development of multiple commercial cultivars with resistance to *Foa*R2 (Daugovish et al., 2008). PI 181714 is currently listed by the USDA as *A. graveolens* without a variety designation; we have never observed a var. *rapaceum*-type of enlarged hypocotyl, and it is morphologically consistent with var. *secalinum*. Regardless, although we identified *A. graveolens* PI 181714 as a source of resistance to *Foa*R4, very few other accessions were promising (Supporting information, Table S1). This dearth of genetic material indicates that more extensive conservation of wild *Apium graveolens* var. *graveolens* (Frese et al., 2018) and var. *secalinum* from the Mediterranean basin could be a critical resource for controlling the new pathogens and pests of *A. graveolens* that will arise in the future.

5 | CONCLUSIONS

Resistance to *Foa*R4 and *Foa*R4 in F_1S_2 and F_1S_3 that were derived from a 'Challenger' X *A. graveolens* PI 181714 will be useful for celery breeding and eventual control of *Foa*R4.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Lynn Epstein and Sukhwinder Kaur designed the experiments. Sukhwinder Kaur was primarily responsible for conducting the experiments. Lynn Epstein analysed the data and wrote the manuscript with input from Sukhwinder Kaur.

DATA AVAILABILITY STATEMENT

Aliquots of 50 seeds of the three F1S2 and PI 181714 and 25 seeds of F1S3 76–8–36-124 can be requested by e-mail to lepstein@ucdavis.edu. The data that support the findings in this study will be available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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