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Seed Density Classification Influences Germination and Seedling Growth of Cotton[1]

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ABSTRACT

Among cotton (*Gossypium hirsutum* L.) seeds with densities less than 1.00 g cm^{-3}, germination performance has been shown to be proportional to the seed density. Since cotton seeds produced in the mid-South usually attain densities higher than 1.00 g cm^{-3}, we evaluated the germination performance of seeds with densities ranging from $\leq$ 1.00 to 1.10 g cm^{-3} to determine if the proportionality between seed density and germination performance might extend beyond 1.00 g cm^{-3}. In laboratory, greenhouse, and growth chamber studies, maximum germination and sustained seedling growth were produced by seeds with initial densities of 1.04 and 1.06 g cm^{-3}. The quantity of oil in the seeds was increased with seed density, but both seed weight and seed volume were decreased in the higher density seed classes. The ratio between seed oil and seed protein contents increased through a seed density of 1.06 g cm^{-3}, then decreased slightly. Germination and seedling growth exhibited the same response to density classification as did the oil:protein ratio. The principal deficiency of the high density cotton seeds appeared to be the low weights of cotyledonary reserves available for mobilization during germination. Selection of cotton seeds on the basis of density should therefore be done so that the small volume, light weight, but high density seeds are eliminated with the low density seeds.

Additional index words: ***Gossypium hirsutum*** **L., Seed quality, Seed oil, Seed protein, Seedling vigor.**

A CONSISTENT problem in the production of cotton (*Gossypium hirsutum* L.) has been the variable performance of seeds within a given seedlot. This variation is often exhibited by the failure to establish a stand, the establishment of an uneven stand, and by the nonuniform growth of seedlings. Wanjura et al. (1969) have demonstrated the desirability of rapid, uniform establishment of a stand of vigorously growing cotton seedlings. They found that the majority of the yield was produced by those plants originating from seedlings that had emerged most quickly from the soil.

Variable performance of cotton seeds has stimulated considerable research for procedures to facilitate the selection of vigorous, high quality seeds from a seedlot. Chester (1938) initially reported differential performance of cotton seeds graded into contrasting density groups and related these differences to the level of infection by seed-borne disease organisms. Later, Arndt (1945) identified a significant environmental component of the mean seed density of a cultivar but indicated that cultivars did tend to rank similarly among environments. Tupper et al (1970, 1971) found that seed density was directly related to the earliness of germination, and that the positive effects of density were more important than seed weight in 7-day germinator evaluations. Similarly, Ferguson and Turner (1971) observed that the degree of fill was positively related to both emergence and seedling vigor. Turner and Ferguson (1972) later associated seed fill with early crop maturity. Subsequent work (Minton and Supak, 1980) indicated that medium density seeds were superior in terms of germination, emergence, disease incidence, and lint yield. Hess (1977) reported effective genetic selection to increase lint yield by increasing seed density. Coincident with the enhancement of seed density, however, both seed index and seed volume decreased. In laboratory evaluations of density-classified cotton seeds produced in the Mississippi Delta, King and Lamkin (1979) reported that optimal early germination performance was obtained with seeds of densities between 1.04 and 1.08 g cm^{-3}

Working with seeds of densities generally less than 1.00 g cm^{-3}, Bartee and Krieg (1974) obtained a complete compositional analysis of four density classes. The predominant changes were in the concentrations of lipid, N (protein), and carbohydrate; each increased as seed density increased. Krieg and Bartee (1975) then evaluated the influence of seed density on various aspects of germination and emergence; they found that the initial rate of water uptake and the leaching of salts during imbibition were inversely proportional to seed density. Krieg and Carroll (1978) demonstrated that seed maturity (density) and seed weight determined seedling growth rate, and that initial radicle growth rates were proportional to lipid utilization at low temperatures.

Generally, these studies have been conducted with seeds produced in west Texas, where mean seed densities apparently tend to be low. Seeds produced in the mid-South, however, develop under different environmental conditions and usually attain densities somewhat higher than do seeds produced in west Texas. Since this higher mean seed density might influence the seedlot performance, we undertook an evaluation of cotton seeds produced in the mid-South to determine the effects of this higher level of seed density on both germination and seedling growth.

MATERIALS AND METHODS

Seeds of the cotton cultivar 'Stoneville 213' and of a glandless derivative of the cultivar 'Stoneville 7A' were evaluated in these studies; all evaluations were made during the first season after seed production. The Stoneville 7A glandless seeds were developed through five generations of backcrossing to the cultivar; the seeds evaluated in this study were from an advanced generation of the original seed stock. The Stoneville 213 seeds were obtained commercially while the Stoneville 7A glandless seeds were produced in experimental plots at Stoneville, Miss. Seeds were separated into density classes by the sucrose floatation procedure described by Hess (1977) and King and Lamkin (1979). Sucrose solutions ranged from 0 to 25% (w/v), in increments of 5%. Seeds were transferred from one concentration to the next higher concentration of sucrose if they did not float in the lower-density solution. All seeds that floated in any sucrose solution were removed, rinsed, and dried. Upon completion of the density classification, six lots of seeds had been produced. These are identified, by density, as follows: I $\leq$ 1.00 $<$ II $\leq$ 1.02 $<$ III $\leq$ 1.04 $<$ IV $\leq$ 1.06 $<$ V $\leq$ 1.08 $<$ VI $\leq$ 1.10 g cm^{-3}. The distribution of seeds among the intermediate classes approximated a normal curve.

Compositional characteristics of Stoneville 213 seeds were determined in triplicate on samples from each density class. Weights and volumes were determined on 100-seed samples; volumes were

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measured by volumetric displacement of methanol. Protein and oil were determined by infrared reflectance from ground cotton seed meal. Reflectance was measured by a Neotec GQA-31EL[3] that had been calibrated for cottonseed by the methods described by Rinne et al (1975).

Imbibition was measured on seeds of density classes I, III, and V of Stoneville 7A glandless. Samples of 25 seeds of each density class were incubated in aluminum tea balls in an aerated 30 C water bath. Periodically during the incubation, seeds were removed from the water bath, drained, blotted free of surface moisture, weighed, and returned to the water bath. A simultaneous evaluation was made of the imbibition by seeds of these three classes from which the seedcoats had been removed prior to incubation. These experiments were conducted twice, with five replications per time. The combined data for changes in fresh weight of the seeds were analyzed to determine density class differences for imbibitional water absorption.

Germination and seedling growth were measured with Stoneville 213. Early germination effectiveness was evaluated in a dark 30 C germinator. Seeds of density classes I, III, and V were placed on moist blotter paper in petri dishes and placed into the germinator. Seedlings were harvested after 2, 5, and 7 days, then separated into cotyledon, hypocotyl, and root sections. These tissues were dried 24 hours at 75 C, then weighed. Analyses of variance were conducted on the data collected from 10 replications of this study to determine the significance of the density effects on early seedling growth.

Seedling growth was also measured in greenhouse evaluations. Seeds of all six density classes were planted approximately 2 cm deep in a 1:1 mixture of sandy loam and sand in 10 cm plastic pots. After planting was completed, the pots were sub-irrigated and placed on greenhouse benches. Seedlings were harvested 7 and 14 days after planting, then separated into cotyledon, stem, and root sections, dried at 75 C, and weighed. Greenhouse evaluations were replicated eight times.

Longer term evaluations were conducted in growth chambers set to produce a 12 h photoperiod at a photosynthetic photon flux density of 250 μmol m^{-2} sec^{-1} and 32 C, with a night temperature of 21 C. Seeds were planted in 9.2 cm plastic cups filled with a 1:1 mixture of Jiffy mix[3] and sand. After emergence (3 days after planting), plants were irrigated weekly with Peters' liquid fertilizer[3]. Seedlings were harvested weekly, beginning 1 week after emergence and continuing through four harvests. Harvested material was separated into cotyledon, leaf, shoot (aerial stem), and root sections. All tissues were dried 48 hours at 70 C before they were weighed. Two chamber tests were conducted with three replications in each test.

RESULTS AND DISCUSSION

Both the size and chemical composition of Stoneville 213 seeds were significantly affected by density (Fig. 1 a, b). High density seeds weighed less and occupied less volume than did the low density seeds. Since the relative change in volume was greater than the change in weight, the volume reduction was the major contributor to the observed increase in seed density. Seed volume is established during the first 3 weeks of development, while most seed weight accumulates from the fourth through the sixth week of development (Leffler, 1981). These changes in volume and weight suggest that the high density seeds had initiated development during a period of nutritional stress that subsequently lessened during the seed filling period. Such a pattern would likely be observed during and just after peak bloom, when the competitive sink demands of other developing fruit would be greatest.

Significant differences for oil concentration, but not for protein concentration, were found among density classes of Stoneville 213 seeds (data not shown). Comparing seeds of densities less than 1.00 g cm^{-3}, Bartee and Krieg (1974) found that both oil and N (protein) percentages increased with seed density. Our data indicate that this dual increase might continue only through a density of about 1.04 g cm^{-3} (density class III), with the principal change being in the oil concentration. When the compositional data were converted to a weight-per-seed basis (Fig. 1 b), maxima for these constituents were observed in density classes II (protein) and III (oil). This differential response between oil and protein to the density classification produced a continual increase in the oil:protein ratio through seed density class IV.

In most species, seed density classification results in the selection of high-protein seeds in the high density classes; this pattern is consistent with the relative densities of protein and oil. Both Hartwig and Collins (1962) and Smith and Weber (1968) successfully used density classification in making selections in their soybean improvement programs. That this does not hold true for cotton seeds reflects the structural differences between cotton seeds and other seeds such as soybeans. The degree to which the embryo fills the seedcoat affects the density of cotton seeds to a greater extent than does the storage product composition of the embryo. This degree of fill is determined by the conditions existing

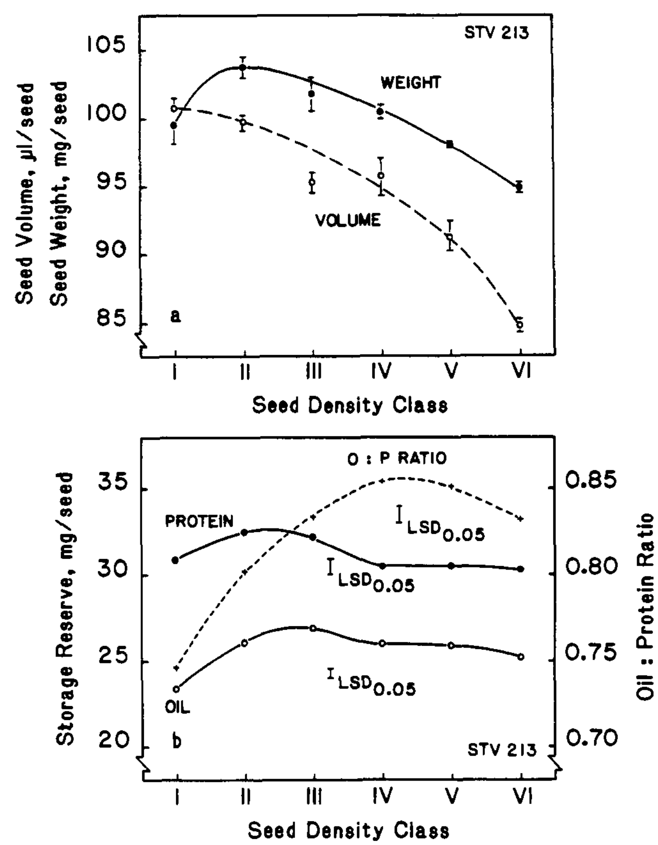

Fig. 1. a. Volumes and weights of Stoneville 213 cotton seeds separated into density classes. Bars at each data point represent the standard error of that mean. b. Storage product composition of cotton seeds separated into density classes.

[3] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

during the periods of seed development, while the composition of the seed more directly reflects the relative temporal periods of oil and protein accumulation. Since the deposition of oil in the embryo occurs later than that of protein (Leffler, 1981), external factors producing a differential storage product accumulation preferentially affect the deposition of oil. Low density cotton seeds have generally been found to be poorly filled, with embryos enclosed within seedcoats that occupy a relatively large volume. High density seeds, conversely, are usually well filled, but with embryos that are restricted in both volume and weight.

During the initial 8 hours of water uptake by Stoneville 7A glandless cotton seeds, the influence of initial seed density (class I vs. classes III and V) became significant within 3 hours and remained significant thereafter (Table 1). Removal of the seedcoats had a marked influence upon the amount of water absorbed, but there was little evidence in the analysis of variance of an interaction between the seedcoat status and the seed density. Inspection of the means suggested, however, some interesting trends, so both seed density and seedcoat status class means are presented. These analyses indicate that the observed differences in hydration by seeds of these densities were probably not secondary effects attributable to the seedcoat properties.

Table 1. Weights of Stoneville 7A glandless cotton seeds during imbibition at 30 C.

Seed group comparison	Hours of imbibition						
	'0'†	1	2	3	4	6	8
	mg·seed⁻¹						
Density class							
I	77.0 b‡	105.6 b	122.4 b	131.9 b	135.7 b	142.7 b	146.3 b
III	88.7 a	122.3 a	137.2 a	145.6 a	149.0 a	154.6 a	158.8 a
V	90.4 a	123.8 a	136.2 a	145.2 a	149.7 a	155.5 a	158.2 a
Seedcoat status							
Intact	99.6 a‡	120.1 a	140.3 a	155.9 a	161.0 a	167.9 a	171.6 a
Removed	71.2 b	114.4 a	123.5 b	125.8 b	128.5 b	133.9 b	137.3 b

† Seeds were immersed in water for 5 min before they were weighed.
‡ Within a comparison at any time, means followed by the same letter are not significantly different as determined by Duncan's New Multiple Range Test, p = 0.05.

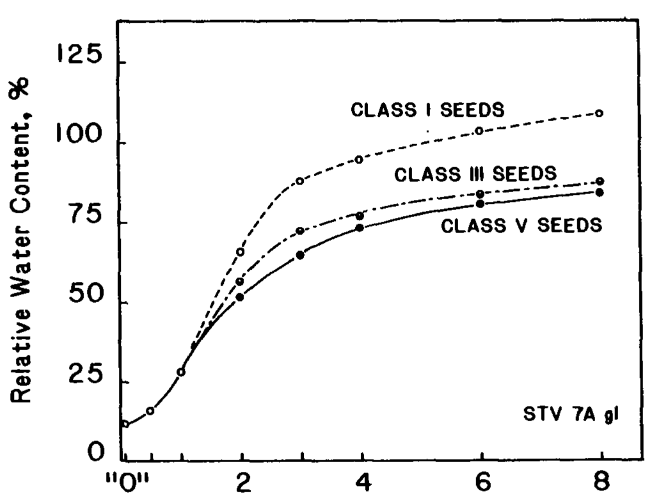

Fig. 2. Relative water contents of low, medium, and high density Stoneville 7A glandless cotton seeds during imbibition at 30 C.

After 2 hours of imbibition, seeds of density class I had attained a higher relative water content than had the seeds of density classes III and V (Fig. 2). This differential hydration was sustained for the 8 hours duration of the study. No significant differences were detected for the relative water contents of seeds of density classes III and V. These data are in general agreement with the results of Krieg and Bartee (1975), who found that water uptake was inversely proportional to density, among lower density seeds.

Germination by Stoneville 213 seeds of each density class was examined both in a 30 C germinator and in the greenhouse. After 3 days in the germinator, the differential germination by seeds of these density classes was found to be highly significant (Fig. 3). Similarly, a greenhouse evaluation of seedling emergence at 5 days after planting showed that significantly more seedlings had emerged from the class III seeds than had emerged from either class I or class V seeds (data not shown). Although the class III seeds sustained a higher level of emergence, later counts showed that density class differences decreased and were no longer significant.

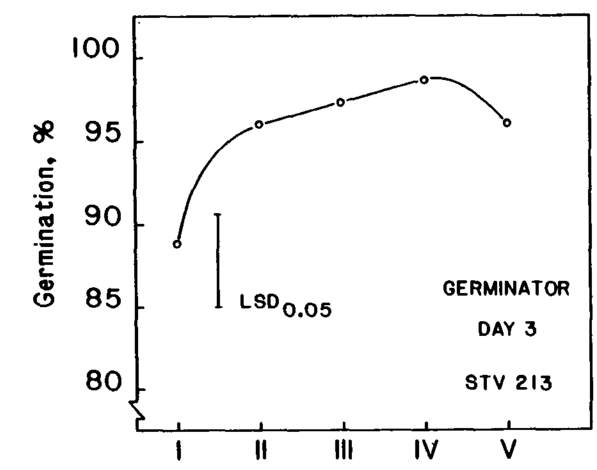

Fig. 3. Stoneville 213 cotton seed germination after 3 days at 30 C, as affected by seed density classification.

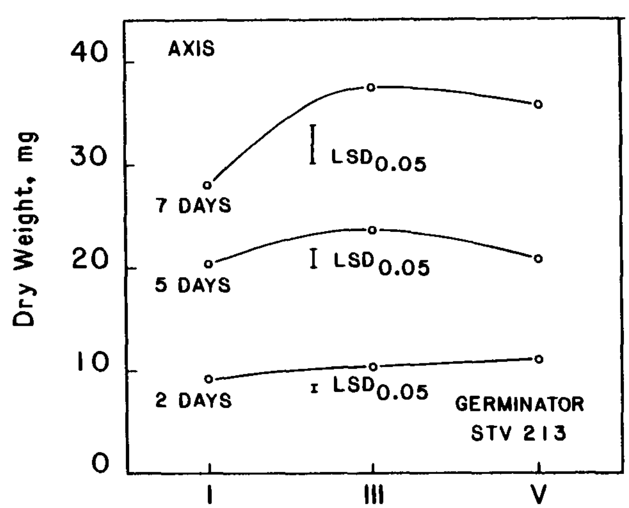

Fig. 4. Axis weights from Stoneville 213 cotton seeds of low, medium, and high density after 2, 5, and 7 days of germination.

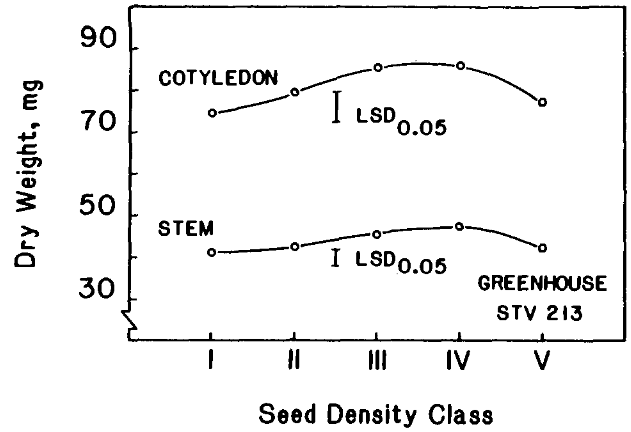

Fig. 5. Stem and cotyledon weights of 14-day greenhouse-grown Stoneville 213 cotton seedlings, as influenced by seed density classification.

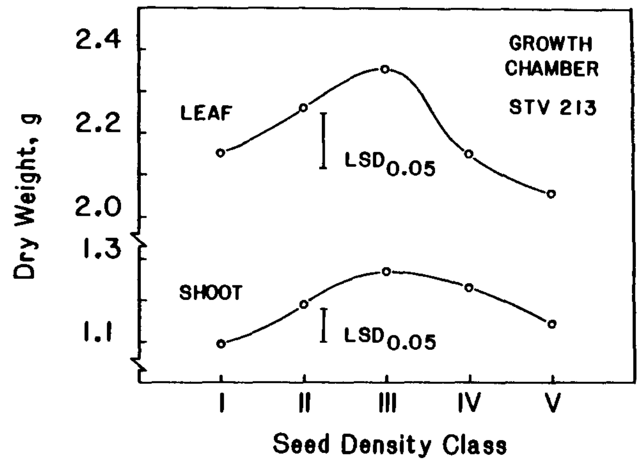

Fig. 6. Shoot and leaf weights of 28-day chamber-grown Stoneville 213 cotton seedlings, as influenced by seed density classification.

Seedlings from class I, III, and V seeds, harvested after 2, 5, and 7 days of germination, exhibited differential growth (Fig. 4). After 5 days, axes of seedlings from class III seeds were significantly larger than those from either of the other two density classes. By day 7, seedlings from both class III and class V seeds were significantly larger than those from Class I seeds. Similarly, Bartee and Krieg (1974) found that radicle growth was positively related to initial seed density classification.

Both greenhouse and growth chambers were used to evaluate seedling growth beyond the initial germination period. The largest seedlings after 14 days in the greenhouse were produced by seeds of density class IV; significant differences due to seed density classification were found for both stem and cotyledon weights (Fig. 5). Cotyledonary expansion was proportional to the cotyledon weight. The cotyledons with the largest area, 23.6 cm^2, were on seedlings from density class IV; those with the smallest, 20.1 cm^2, were on seedlings of density class I. Other plant parts were also analyzed at both harvests; they exhibited a similar pattern of response to the initial seed density classification. These data were somewhat more variable, however, and the differences were not significant, so they are not presented. In longer-term growth chamber evaluations, a similar pattern of response to the seed density classification was observed at each harvest. After 3 weeks of growth in the chambers, the differences among density classes were significant. Maximum seedling growth after 4 weeks in the growth chambers was exhibited by seedlings of seed density class III (Fig. 6). As was noted above for cotyledonary expansion, leaf areas were proportional to the leaf weights. There was a slight but non-significant tendency for the leaves of class III seedlings to have a lower area:weight ratio than those of seedlings from either lower or higher seed density classes.

When expressed as milligram of water per milligram of seed, all seed density classes absorbed equivalent amounts of water during imbibition. The differences in initial seed weights among the density classes created a differential degree of hydration among the classes of seed. Both in our study and in that of Krieg and Bartee (1975), lower density seeds absorbed relatively more water, but the higher density seeds were more effective in the initiation of germination. Clearly, the quantity of water absorbed during imbibition is not directly related to the efficiency of germination initiation. The data of Tupper et al. (1970, 1971) indicate that the beneficial effects of high density seeds are manifested in the rate of initiation of germination. The relatively slower uptake of water by the higher density seeds appears to be associated with a regulated balance among the events that lead to this initiation.

Once germination occurs and seedlings emerge from the soil, a sustained vigorous growth rate becomes of primary importance. The data from our study show that the seeds in density classes III and IV were capable of sustaining, through at least a 4-week period, seedling growth more vigorously than were seeds of either lower or higher densities. These medium density seeds were not only more efficient in the initial onset of germination, but they could apparently mobilize more reserves to nourish the growth of seedlings. Krieg and Carroll (1978) concluded that maturity (density) and seed weight (quantity of available sustrate) determined seedling growth rates at optimum temperatures—such as were used in this study. They also found that at minimum temperatures the initial growth rates were directly related to the lipid consumption. In this regard, the oil:protein ratio may be assigned some importance, as it is the only compositional parameter measured that paralleled the expressed seedling growth. Additionally, a high oil:protein ratio would indicate a relatively high energy content in the seeds, which would be advantageous during germination.

Seedlings from low density seeds not only emerge slowly, they also exhibit low levels of seedling vigor (Ferguson and Turner, 1971; Krieg and Bartee, 1974; Tupper et al, 1970, 1971). Our data indicate that the growth superiority expressed in the very early seedling stage is sustained throughout the first month of vegetative development, and that this differential increases with time. Investigations of seed-density effects in field situations (Minton and Supak, 1980; Turner and Ferguson, 1972) have shown that density, or fullness, ultimately influenced both disease tolerance and productivity of cotton. The productivity contrasts described by Wanjura et al (1969) may therefore be explainable by density differences among seeds within their initial seedlot.

The data collected in our study, while in basic agreement with those of previous reports, extend and amplify those published earlier. Our seedlots were composed of higher density seeds than those generally used in previous studies, and our seedling growth evaluations were conducted for longer periods than has been customary. Because of the higher mean seed density, our study identified a probable optimum for seed density classification in that the light weight seeds among the highest density classes do not sustain maximum seedling growth. Maximum seedling weights occurred in seedlings originating from seeds of density classes III and IV. Above these density levels, the quantities of storage products in the seeds were apparently inadequate to sustain high rates of growth. Our data further indicate that those seedlings that initially develop a growth advantage maintain and increase that advantage throughout the first month of vegetative development. In addition, the ratio between the contents of oil and protein in the seeds has been identified as the only measured seed compositional parameter that paralleled the seedling growth response to seed density classification.

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