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Natural incidence of black point (*Alternaria* sp.) in a group of elite advanced bread wheat (*Triticum aestivum*) lines

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Abstract

One hundred and sixty eight elite advanced bread wheat lines were evaluated for resistance to black point during the crop season 2014-2015 at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Mexico. Sowing dates were November 19 and 28, and December 8, 2014. Harvest and threshing were done manually in ten spikes per line, and the evaluation by visual inspection, counting healthy and infected grains to calculate the percentage of infection. The range of infection for the first sowing date was 0 to 34.6%, with an average of 2.7; sixty lines did not show infected grains; for the second date it was 0 to 43.6%, with an average of 3.1%; fifty lines did not show infected grains; while for the third date, it was 0 to 14.7%, and seventy one lines did not show infected grains. Five lines did not show infected grains in the three dates. Lines with the highest average percentage of infection were: WAXWING/KRONSTADF2004//2*FRNCLN with 18.6%, PFAU/MILAN/5/CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/VEE#7/BOW/4/PASTOR/6/WHEAR/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/C80.1/3*BATAVIA//2*WBLL1/7/C80.1/3*BATAVIA//2*WBLL1/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES with 16.0, and WBLL1*2/BRAMBLING//SAAR/2*WAXWING/4/PBW343*2/KUKUNA//KRONSTAD F2004/3/PBW343*2/KUKUNA with 15.4%. Within the infection categories based on the average of the three dates, one hundred and twelve lines were within the 0.1-2.5% infection category, thirty one within 2.6-5.0%, thirteen within 5.1-10.0%, and seven lines within 10.1-30.0%. The infection average of the group was 2.3% with a range of 0 to 18.6%, but some lines showed high percentage of infection in the dates, like PFAU/MILAN/5/CHEN/AEGILOPSSQUARROSA(TAUS)//BCN/3/VEE#7/BOW/4/PASTOR/6/WHEAR/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/C80.1/3*BATAVIA//2*WBLL1/7/C80.1/3*BATAVIA//2*WBLL1/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES which showed 43.6% in the second date.

Keywords: *Triticum aestivum*; Elite bread wheat; Black point; *Alternaria* spp.

1. Introduction

Many fungal species, including *Alternaria* spp., *Fusarium* spp., and *Helminthosporium* spp. can be isolated from newly harvested wheat grain (*Triticum* spp.), particularly in humid field environments, where they may infect seed when the relative humidity exceeds 90% and seed moisture content 20% [1]. In a study reported in 2018 [2], 21 strains representing 11 genera of fungi were isolated from wheat seed. Black point (BP) or kernel smudge is favored by rainfall during seed maturation, as well as humid weather prevailing for a few days prior to harvest [3,4]; expanding green kernels are most susceptible. BP also promotes premature seed senescence because many of the fungi are saprophytic [1]. *Alternaria alternata* (Fries: Fries) von Keissler and *Bipolaris sorokiniana* (Sacc.) Shoemaker are generally considered the primary causal agents of the disease [5]. The disease is more noticeable around the embryo end of seed (Adlakha and Joshi, 1974; Hanson and Christensen, 1953; Rana and Gupta, 1982; cited by Mathur and Cunfer [5] with a dark

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brown to black color (Figure 1). Severe infection causes discoloration and shriveling of the whole seed [5]; the discoloration may also occur near the brush, in the crease or any part of the seed.

Figure 1 Symptoms of black point caused by the fungus *Alternaria* spp. on the wheat grain

It may be light or dark or with a distinct margin. Vertical sections of the endosperm may present brown to black spots [6]. The disease known as red smudge caused by *Pyrenophora tritici-repentis* (Died.) Drechs., may also be present in seed affected by BP [7]. In addition to the economic loss caused by the degradation and quality of flour and semolina, BP also affects seed germination, and inhibits seedling growth [2]. In the southern region of the state of Sonora in Mexico, BP is an endemic disease of durum (*Triticum durum* Desf.) and bread wheat (*Triticum aestivum* L.), and it also affects triticale (X *Triticosecale* Wittmack) [1,8]. The incidence of the disease under natural conditions varies from crop season to another, and therefore, there is no consistent data on the reaction of germplasm to the disease in order to do the appropriate selection. Conner and Thomas [9] reported that inoculation of germplasm by injection or by vacuum infiltration using the fungus *Alternaria alternata* (Fr.:Fr.) Keissl., are useful methods for the identification of resistant and susceptible germplasm, while Kai-Ge *et al*. [2] were able to complete Koch´s postulates by spraying the inoculum over wheat plants under greenhouse conditions. The objective of this work was to evaluate the reaction of elite bread wheat advanced lines to black point during the crop season 2014-2015 under natural conditions of infection.

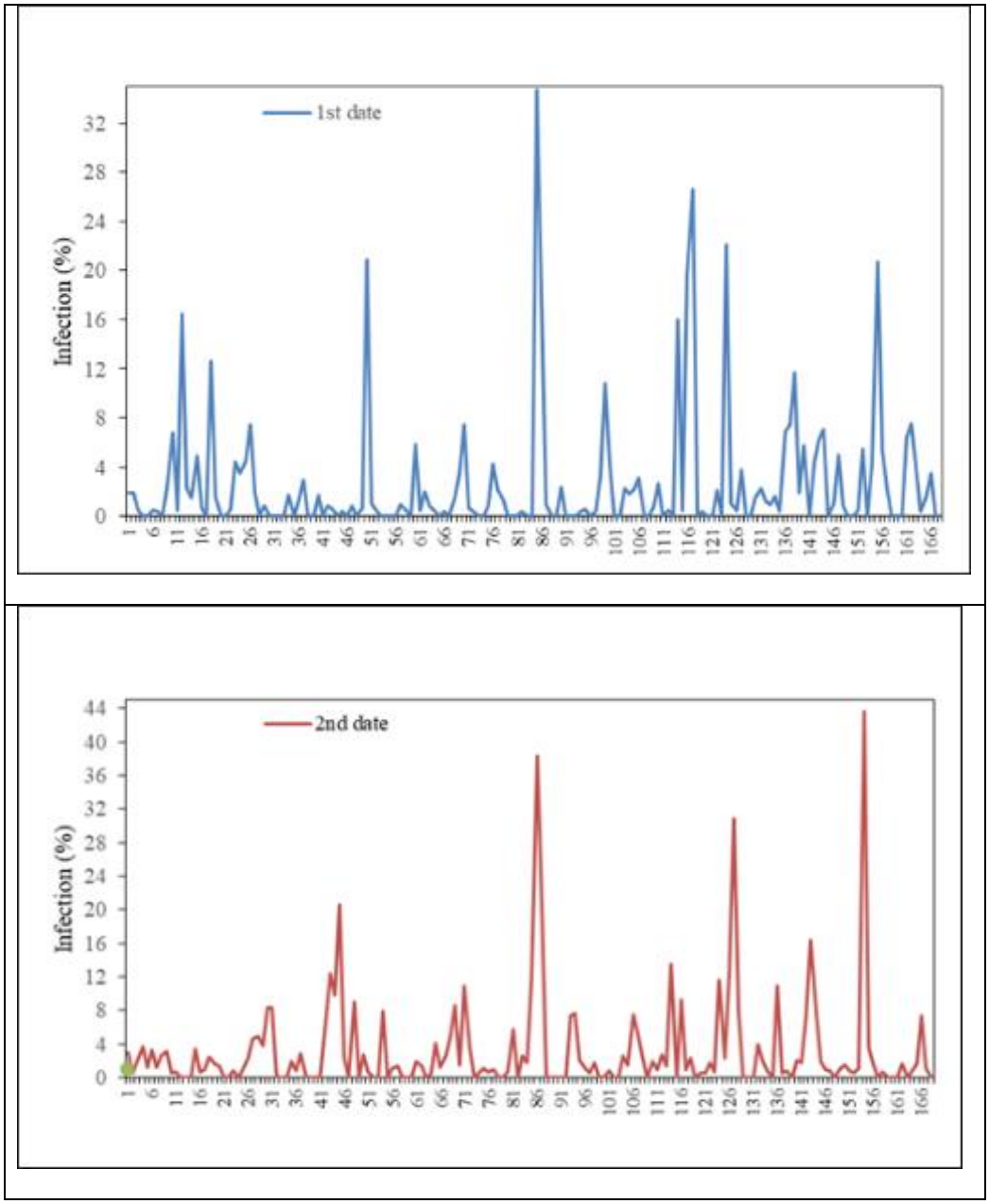
2. Materials and methods

One hundred and sixty eight elite advanced bread wheat lines were evaluated for resistance to black point during the crop season 2014-2015 at the Norman E. Borlaug Experimental Station which belongs to the National Institute for Forestry, Agriculture, and Livestock Research (INIFAP) located in block 910 of the Yaqui Valley in the state of Sonora, Mexico (27º22´3.01" N, 105º55´40.22" W), during the crop season fall-winter 2014-2015. Sowing dates were November 19 and 28, and December 8, 2014, in a clay soil with pH 7.8. Approximately 8 g of seed were used for a bed 0.7 m long in a single row, and without replications. Fertilization consisted of 150 kg ha^{-1} of urea before sowing. An irrigation for seed germination and three complementary ones were applied during the season; 100 kg ha^{-1} of urea were applied just prior to the first complementary irrigation. Thirty days after sowing, the herbicides Topik gold (clodinafop-propargyl) and Situi® xl (metsulfuron methyl) were applied at the rates of 750 mL and 25 g ha^{-1} of commercial product. The harvest was carried out manually and the evaluation by visual inspection, counting the number of infected and healthy grains in ten spikes per line in order to determine the percentage of infection; the lesion size was not taking into consideration. The elite advanced lines evaluated, were produced by the collaborative breeding program between the International Maize and Wheat Improvement Center and INIFAP.

3. Results and discussion

The range of infection for the first sowing date was 0 to 34.6% with an average of 2.7 (Figure 2); sixty lines did not have infected grains, 66 lines fell within the 0.1-2.5% infection category, 17 lines within 2.6-5.0%, 13 lines within 5.1-10%, 11 lines within 10.1-30%, and 1 line showed more than 30.1% infected grains. The range of infection for the second date was 0 to 43.6% with an average of 3.1; fifty lines did not have infected grains, 68 lines fell within the 0.1-2.5% infection category, 20 within 2.6-5.0%, 17 lines within 5.1-10%, 10 lines within 10.1-30%, and three lines showed more than 30.1% infected grains. The range of infection for the third sowing date was 0 to 14.7% with an average of 1.1;

seventy one lines did not have infected grains, 78 lines fell within the 0.1-2.5% infection category, 12 lines within 2.6-5.0%, 4 lines within 5.1-10%, and 3 lines within 10.1-30%. Some lines showed consistency in their reaction to black point: five lines did not show any infected grains in the three dates, eleven showed a range of infection between 0.1 and 2.5%, forty one lines had no infection in two dates and a percentage of infection in the range already mentioned in one date, and thirty four had no infection in one date and similar percentage of infection as the previous group but in two dates. Those lines with high percentage of infection in two dates were No. 85 with a range of 11.6 to 34.6, No. 86 with 17.7 to 38.3, and 124 with 11.6 to 22.0%, although the first two lines in the remaining date had no infected grains, while the third one showed 3.7%. Lines with the highest average percentage of infection were 86 (WAXWING/KRONSTADF2004//2*FRNCLN) with 18.6, 154 (PFAU/MILAN/5/CHEN/AEGILOPSSQUARROSA(TAUS)//BCN/3/VEE#7/BOW/4/PASTOR/6/WHEAR/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/C80.1/3*BATAVIA//2*WBLL1/7/C80.1/3*BATAVIA//2*WBLL1/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA(213)//PGO/4/HUITES) with 16.0, 85 (WBLL1*2/BRAMBLING//SAAR/2*WAXWING/4/PBW343*2/KUKUNA//KRONSTADF2004/3/PBW343*2/KUKUNA) with 15.4, 124 (PICAFLOR #1/NELOKI), 127 (MUNAL#1*2/4/HUW234+LR34/PRINIA



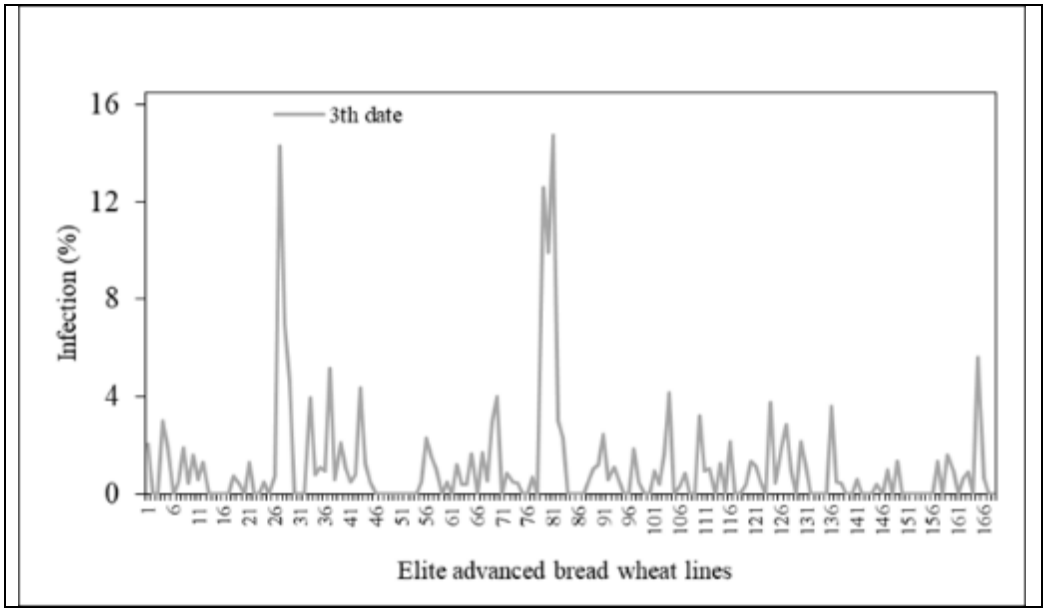


Figure 2 Percentage of infection with black point of 168 elite advanced bread lines, evaluated under natural conditions in three sowing dates at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico, during the crop season 2014-2015

//PBW343*2/KUKUNA/3/ROLF07) with 12.4, 116 (TRCH/7/TUKURU//BAV92/RAYON/6/NG8201/KAUZ/4/SHA7 //PRL/VEE#6/3/ FASAN/5/MILAN/KAUZ) with 10.4, and 114 (CHWINK/3/ROLF07/YANAC//TACUPETOF2001/ BRAMBLING) with 10.2%. The average infection of each line in the three dates are shown in Figure 3, and lines that did not have any infected grain in the three dates are shown in Table 1.

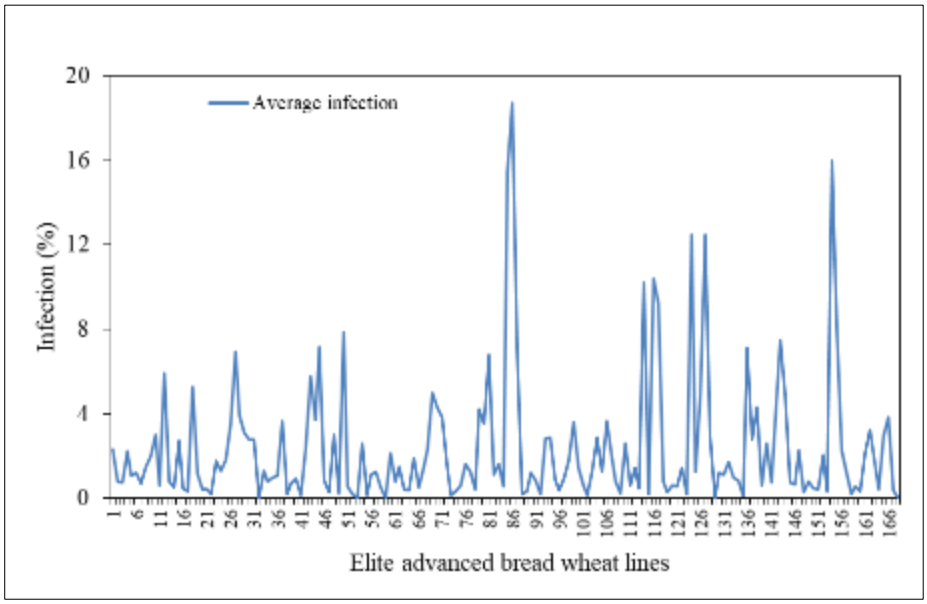


Figure 3 Average percentage of infection with black point of 168 elite advanced bread lines, evaluated under natural conditions in three sowing dates at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico, during the crop season 2014-2015

Table 1 Elite advanced bread wheat lines that did not have any infected grains with black point (*Alternaria* sp.), and that were evaluated under natural conditions in three sowing dates, during the crop season 2014-2015, in the Yaqui Valley, Sonora, México.

No.	Pedigree	Selection history
1	MILAN//PRL/2*PASTOR/4/CROC_1/AE.SQUARROSA (213)//PGO/3/BAV92/5/PAURAQ	CMSA09M00542S-050ZTM-0NJ-099NJ-15WGY-0B
2	FRNCLN*2/KINGBIRD#1	CMSS09B00699T-099TOPY-099M-099Y-7WGY-0B
3	ROLF07/KINGBIRD#1//MUNAL#1	CMSA09M00147T-050Y-050ZTM-050Y-2WGY-0B
4	BECARD #1*2/KINGBIRD#1	CMSS09B00802T-099TOPY-099ZTM-099NJ-099NJ-17WGY-0B
5	NELOKI/CIRNOC2008	CMSS10Y00594S-099Y-099M-4RGY-0B

Within the infection categories of the group based on the average of the three dates, five lines did not show any infected grains, 112 lines fell in the 0.1-2.5% category, 31 lines in 2.6-5.0%, 13 lines in 5.1-10.0, and 7 in the 10.1-30.0 infection category. The overall average of the group was 2.3% with a range of 0 to 18.6% (Figure 4).

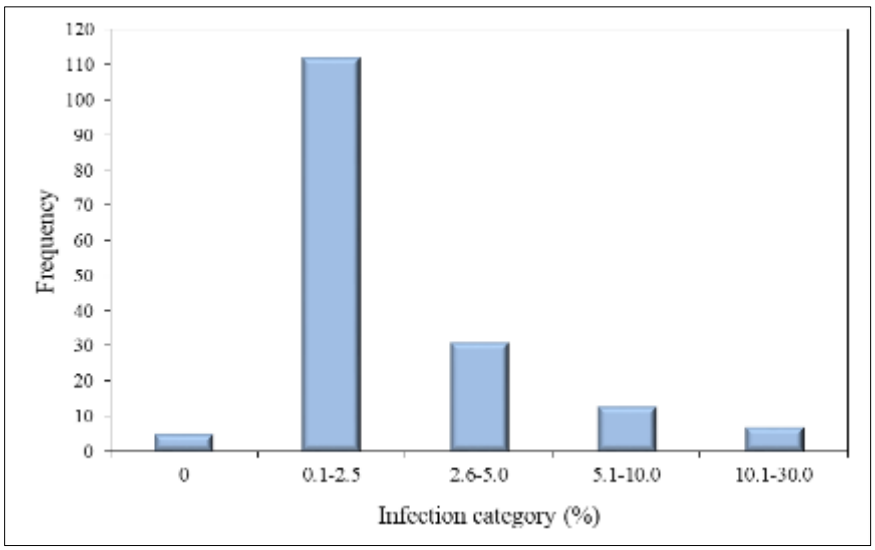


Figure 4 Black point infection category (%) of 168 elite advanced bread lines, evaluated under natural conditions in three sowing dates at the Norman E. Borlaug Experimental Station in the Yaqui Valley, Sonora, Mexico, during the crop season 2014-2015

The incidence of black point is quite variable in each fall-winter crop season in the regions where wheat is cultivated in the south of the state of Sonora, in both bread and durum wheat; this is partly due not only to the weather conditions that prevail during the season, but also, due to the different climatic zones [10,11]. The primary inoculum is another important aspect which will be influenced by weather, as well as the capacity of acting as saprophytes by some fungal species, like *Alternaria triticina*, *Helminthosporium sativum*, and *Fusarium* spp. which may survive in plant debris [1]. Like in the present study, the percentage of infection of black point may be high in some crop seasons: in 2009-2010 the bread wheat lines BABAX/LR42//BABAX/3/ER2000, BABAX/LR42//BABAX*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ, and TC870344/GUI//TEMPORALERAM87/AGR/3/2*WBLL1 had 30.8, 27.3, and 20.53% infected grains, respectively [12]; in crop season 2012-2013, NL1048/4/CHIBIA//PRLII/CM65531/3/SKAUZ/BAV92 showed a maximum of 12.3% infected grains [13]; in 2013-2014, lines SOKOLL*2/3/BABAX/LR42//BABAX and KISKADEE#1/CHYAK both had 31.4% infected grains [14]; and in 2014-2015, the same line SOKOLL*2/3/BABAX/LR42//BABAX had a maximum of 22.6% infected grains [15]. The high percentage of wheat grains infected with black point affects the quality and consequently the value of the products. Therefore, besides aiming

to the use of resistant cultivars [16], black point can be partially controlled by reducing the frequency of irrigation after heading and by reducing nitrogen rates, without affecting the grain yield or its quality. Testing experimental bread wheat germplasm for their reaction to black point should be a continuous effort in order to secure tolerant, and even more resistant genotypes that could be candidates for commercial release. This will not only contribute to improve the economic return for the wheat producers, but it will also be positively reflected in the region and their country.

4. Conclusion

Lines that did not have any infected grains with black point in the three dates were MILAN//PRL/2*PASTOR/4/CROC_1/AE.SQUARROSA(213)//PGO/3/BAV92/5/PAURAQ, FRNCLN*2/KINGBIRD#1, ROLF07/KINGBIRD#1//MUNAL#1, BECARD#1*2/KINGBIRD#1, and NELOKI/CIRNOC2008.

Lines with the highest percentage of infection were PFAU/MILAN/5/CHEN/AEGILOPSSQUARROSA (TAUS)//BCN/3/VEE#7/BOW/4/PASTOR/6/WHEAR/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/C80.1/3*BATAVIA//2*WBLL1/7/C80.1/3*BATAVIA//2*WBLL1/5/REH/HARE//2*BCN/3/CROC_1/AE.SQUARROSA (213)//PGO/4/HUITES with 43.6% in the second date, WAXWING/KRONSTAD F2004//2*FRNCLN with 38.3% in the second date, WBLL1*2/BRAMBLING//SAAR/2*WAXWING/4/PBW343*2/KUKUNA//KRONSTAD F2004/3/PBW343*2/KUKUNA with 34.6% in the first date, MUNAL#1*2/4/HUW234+LR34/PRINIA//PBW343*2/KUKUNA/3/ROLF07 with 30.8% in the second date, and MUNAL#1/NELOKI with 26.6% in the first date.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No conflict of interest.

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