Effect of Matric and Osmotic Potential on Teliospore Germination of *Tilletia indica*

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ABSTRACT

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The interaction of matric and osmotic water potential with temperature on the germination of teliospores of *Tilletia indica* was investigated on several substrates. Water potential was osmotically adjusted with KCl, NaCl, or sucrose, or matrically adjusted with polyethylene glycol 6000. Maximal germination on all osmoticants occurred at the highest potential tested (−1.4 bars); little or no germination occurred below −10.5 bars with NaCl or KCl, or below −14.5 bars with sucrose. Similarly, germination did not occur below −11.0 bars on polyethylene glycol 6000 and was reduced to less than 5% on soil below −15 bars. Optimal (15–20 C) or suboptimal (10 C) temperatures allowed greater germination at lowered osmotic potential compared with supraoptimal temperature (25 C). On pH adjusted soil or water agar, maximal germination occurred from pH 5.0 to 8.8 and was significantly reduced below 4.5.

The Karnal- or partial bunt disease of wheat, caused by *Tilletia indica* Mitra (= *Neovossia indica* (Mitra) Mundkur), was confined until recently to a limited geographical area comprising India, Afghanistan, Pakistan, Iraq, and Nepal (23,34). Its recent occurrence in Sonora, Mexico, has increased concern about the potential for spread into the United States and other countries (2). Therefore, an assessment of the parameters necessary for disease establishment and perpetuation are essential.

The effect of soil moisture on teliospore germination and infection of wheat by other *Tilletia* spp. has been described (7,22,28,29). However, the disease cycle of Karnal bunt is radically different from that of other bunt diseases of wheat. Karnal-bunt infection occurs in the florets of mature wheat plants from wind- or waterborne sporidia, whereas infection by the common and dwarf bunts (*Tilletia caries* (DC.) Tul. or *T. foetida* (Wallr.) Liro, and *T. controversa* Kuhn, respectively) occurs in wheat seedlings from teliospore germination products in or near the soil (8,21,23). Moreover, infection by *T. indica* is localized, whereas infection from other *Tilletia* spp. on wheat is systemic (21,23).

Teliospore germination at the soil surface at the time of spike emergence is a prerequisite for initiation of the Karnal-bunt disease cycle and factors conducive or suppressive to teliospore germination are, in part, responsible for disease incidence and severity. Previous reports showed that maximal teliospore germination (55–60%) occurred after 14–21 days incubation at 15–22 C on water agar or moist soil, and that the teliospores could survive periods of freezing or desiccation (33,34). High disease incidence has been associated with rain although it remains unclear which aspect of the disease cycle is favored by this additional moisture (3,32). This is a report of the effects of water potential on teliospore germination of *T. indica* on several media. A brief report has been published (17).

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MATERIALS AND METHODS

Teliospores. Wheat kernels infected with *T. indica* were collected from the Centro de Investigaciones Agricolas de Noroeste, Cd. Obregon, Sonora, Mexico. The infected kernels were stored at room temperature (23–26 C) and were 12–18 mo old when used. Except where noted, teliospores were removed from infected kernels and surface-sterilized with sodium hypochlorite as previously described (33). Teliospores were applied as a water suspension by pipeting 0.2 ml onto the surface of agar in 60-mm-diameter petri plates. When soil was used, teliospores were either sprayed onto the surface as a water suspension with an air brush or showered dry through nylon cloth. Percent teliospore germination was determined by observing three to five plates per treatment with a light microscope (×100) and counting how many of 200 teliospores per plate germinated. The proportion of germinated teliospores was angular transformed and used in a one- or two-way analysis of variance procedure, followed by Fisher's least significant difference procedure when the F statistic was significant (16). Error bars, in figures where they appear, represent Fisher's LSD ($P = 0.05$).

Soil. The soil used was a Green Canyon gravelly loam (pH = 7.6; lime, 0.4%; total N, 0.7%; Fe, 9.8 ppm; Zn, 8.6 ppm; K, 357 ppm; P, 33 ppm; and total organic matter, 2.5%). Before use, the soil was air-dried and sieved through a 4-mm-mesh screen. A soil moisture release curve was developed by the method of Fawcett and Collis-George (18). Soil moisture content was determined gravimetrically (grams of water per grams of soil dried for 24 hr at 105 C). Except where noted, soil moisture content was adjusted to approximate field capacity (−0.3 bars) with sterile deionized water and 25 g of the soil was added to each petri plate. Soil was either autoclaved twice for 1 hr each on consecutive days or fumigated with methyl bromide. For fumigation, soil (500 g) was placed under vacuum in 2-L flasks, and pure methyl bromide gas was injected until atmospheric pressure was attained. After 24 hr, the flasks were aseptically evacuated five times over 2 days to remove any residual gas before the soil was used. To confirm sterility, soil was placed on potato-dextrose agar and observed after 3 days incubation at 23–26 C.

The effect of shifting temperature regimes on teliospore germination was assessed by incubating plates containing teliospores at 15 or 30 C continuously or by shifting them at intervals of 7 or 14 days to the other temperature. Percent teliospore germination was assessed after 14 and 21 days.

Water agar (WA) was adjusted to various osmotic potentials by the addition of either KCl, NaCl, or sucrose. The total osmotic potential was the sum of the water potential of the WA (−1.4 bars) as determined by dew-point thermocouple psychrometry (Wescor, Inc., Logan, UT) and the osmotic potential of added solutes (31). Germination was assessed in 0.2-ml aliquots of teliospores suspended in either sterile deionized water or aqueous solutions osmotically adjusted to the same potentials as the agar in plates onto which they were placed. Plates were sealed with Parafilm to prevent moisture loss and incubated at 15 C for 2 wk before teliospore germination was assessed.

To study the effect of osmotic potential on rate of teliospore germination, KCl was used to osmotically adjust WA. After seeding with teliospores, plates were incubated at 15 C and percent germination assessed after 5, 6, 9, 12, and 16 days. To assess temperature and osmotic potential effect on teliospore germination, teliospores were introduced on KCl-adjusted WA and placed in incubators at 10, 15, 20, or 25 C, and the percent germination assessed after 2 wk.

To study the effect of matric potential on teliospore germination, two matrically adjusted media were used, polyethylene glycol (PEG) 6000 and soil. A modified method of Brownell and Schneider (9) was used for PEG 6000. Polyester batting (2 cm thick) and tightly woven polyester cloth were cut into 60-mm-diameter disks. The cloth was placed on top of the batting and placed into petri plates. A 20-ml volume of either sterile deionized water or an aqueous solution matrically adjusted with PEG 6000 was added to each plate. Teliospores were showered dry onto the surface of the cloth. Petri plates were sealed with Parafilm, incubated at 15 C, and percent germination assessed after 2 wk.

To study the effect of soil matric potential on teliospore germination, soil was matrically adjusted by adding appropriate amounts of water to soil in polyethylene bags. Soil and water were mixed thoroughly and allowed to equilibrate for 4 days. The soil was then divided into 25-g portions and placed in 60-mm-diameter petri plates. The petri plates were placed into polyethylene bags for 1 day before dusting teliospores onto the soil surface. Plates were placed individually into polyethylene bags incubated at 15 C for 2 wk, and percent teliospore germination assessed.

To study the effect of pH-adjusted soil on teliospore germination and to compare it with pH-adjusted WA, soil was leached with 10 volumes of deionized water to remove soluble salts and the pH altered by the addition of either H_2SO_4 or $Ca(OH)_2$. Soil reaction was measured by the method of Schofield and Taylor (30). Soils were moistened to field capacity and added to petri plates. WA was pH-adjusted as described previously (33). After teliospore introduction on both media, the plates were sealed with Parafilm, placed at 15 C, and the percent teliospore germination assessed after 1 and 2 wk.

RESULTS

The percent germination of teliospores after 2 wk on nonsterile soil was not significantly different from that on autoclave-sterilized soil (Fig. 1). However, germination on methyl bromide-sterilized soil was significantly reduced compared with the nonsterile control. The decrease in teliospore germination on methyl bromide treated soil was possibly due to a toxic effect of residual bromide in the soil following fumigation.

Shifting temperature had a profound effect on teliospore germination (Fig. 2). Teliospores incubated constantly at 15 C germinated to a maximum of 53% after 14 days and decreased about 10% after an additional 7 days. In contrast, teliospores incubated constantly at 30 C showed only a trace of germination after 21 days. However, teliospores incubated at 30 C for 7 or 14 days and then shifted to 15 C began to germinate, whereas those incubated at 15 C for 7 or 14 days and then shifted to 30 C ceased germination. Germination was delayed or arrested by incubation at 30 C and resumed unhindered after this unfavorable period.

Maximal germination of teliospores after 14 days occurred on unamended WA (−1.4 bars; Fig. 3), whereas germination on WA osmotically adjusted with NaCl, KCl, or sucrose was significantly less. Complete inhibition of germination occurred below −11 bars on KCl- and NaCl-amended WA, whereas complete inhibition occurred below −14.5 bars on sucrose-amended WA.

The rate of teliospore germination was also affected by water potential (Fig. 4). On WA osmotically adjusted with KCl, a similar rate and percent germination occurred after 2 wk at −1.4 and −2.6 bars. At −3.7 bars, however, germination was slower, although after 14 days the percent germination was the same as at higher water potentials. Both germination rate and percentage over 14 days was reduced significantly at −6.0 bars and was completely inhibited below −8.3 bars.

The decrease in germination with decreasing water potential was similar at 10, 15, and 20 C (Fig. 5). However, germination was more severely inhibited by low water potentials at 25 C than at lower temperatures; less than 1% germination occurred below −6 bars at 25 C.

On media matrically adjusted with PEG 6000 germination

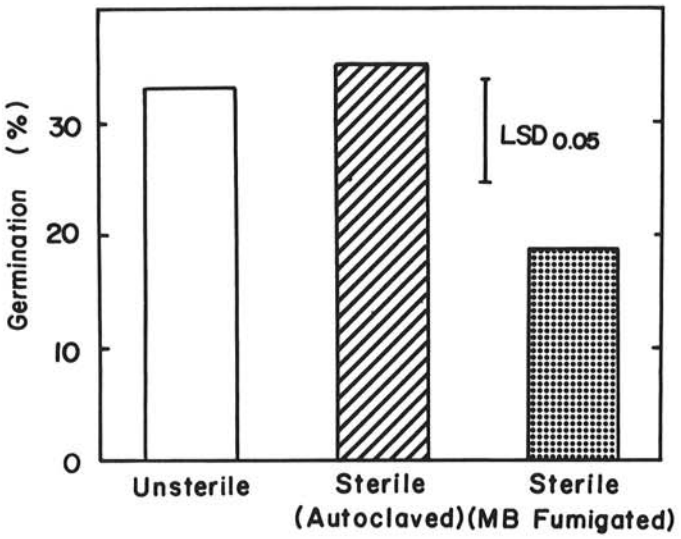

Fig. 1. Germination of teliospores of *Tilletia indica* after incubation for 2 wk on unsterile soil and on autoclaved- and methyl-bromide (MB)-fumigated sterile soil.

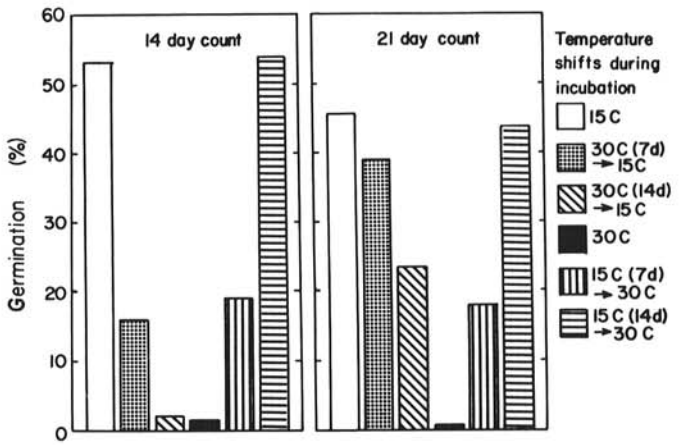

Fig. 2. Germination of teliospores of *Tilletia indica* on water agar after incubation at intervals of 7–21 days at 15 or 30 C. The germination observed at 21 days in the first (continuous 15 C) and last (15 C for 14 days, 30 C for 7 days) treatments was lower because mycelial growth obscured germinated teliospores.

decreased with decreasing matric potential and was completely inhibited below −11 bars (Fig. 6). The decrease in germination was similar to that observed on osmotically adjusted agar. Percent germination after 14 days incubation at 15 C was greatest at zero potential.

On soil, the highest germination (35%) after a 1–2 wk incubation at 15 C was attained at −0.1 bars. On water-saturated (0 bars) soil, germination was only 10%. As soil matric potential decreased from −0.1 bars, teliospore germination also decreased; germination was only 4% at −16.5 bars (Fig. 7).

The effect of pH on germination of teliospores after 7 and 14 days was compared on WA and soil (Fig. 8). After 7 days, percent germination on agar was higher than on soil over the pH range tested. However, after 14 days, germination was comparable on both WA and soil with maximal germination (50–60%) occurring over a pH range of 4.5–8.8.

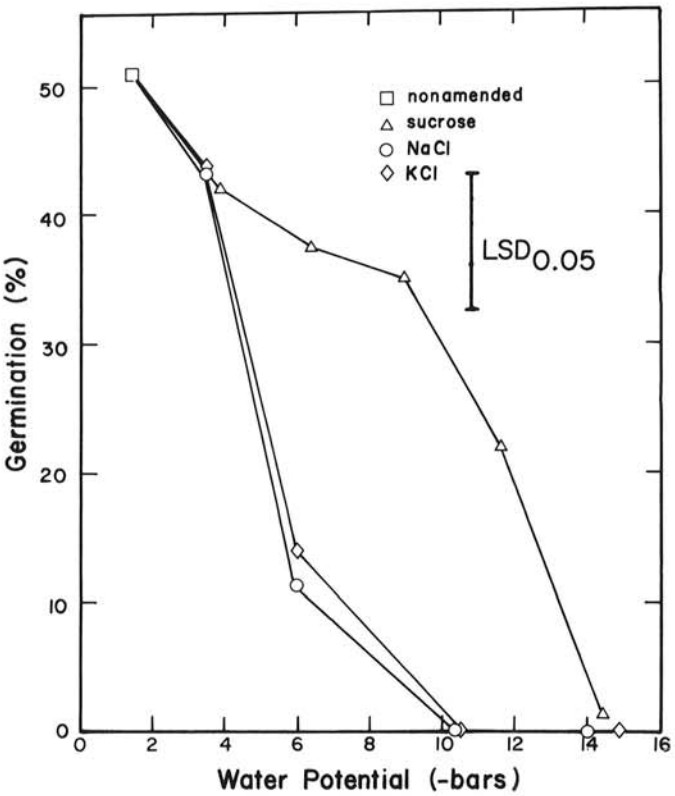

Fig. 3. Germination percentage of teliospores of *Tilletia indica* after 14 days at 15 C on unamended water agar and water agar adjusted to various osmotic potentials with sucrose, CaCl, or KCl.

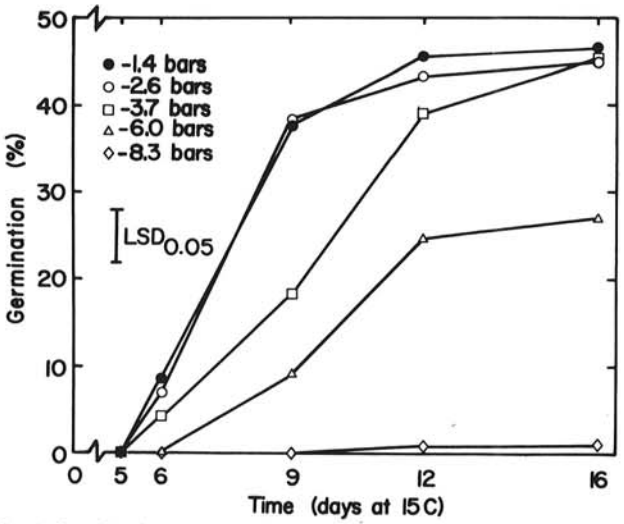

Fig. 4. Germination rate of teliospores of *Tilletia indica* incubated at 15 C on water agar osmotically adjusted with KCl.

DISCUSSION

This is the first report on the influence of osmotic and matric water potential on teliospore germination of the bunt fungi. Teliospore germination was delayed and both the rate and percentage were decreased by decreasing water potentials on several media. Similar inhibitory effects have been observed on the mycelial growth (5,26,31) and spore germination (4,5,12,19) of other fungi. In this study, an increased tolerance of teliospore germination to sucrose-osmotic adjustment was observed (Fig. 3). Although curves relating fungal growth to different osmoticants are generally similar (20), a similar increased tolerance to sucrose-osmotic adjustment in *Sclerotinia borealis* Bub. & Vleug. was reported by Bruehl and Cunfer (10).

The response of *T. indica* to decreasing water potential differed in several respects from that generally reported for many fungi. Teliospore germination was more sensitive to decreasing water potential than spore germination of most fungi that have been investigated (14). Teliospore germination was similar on both

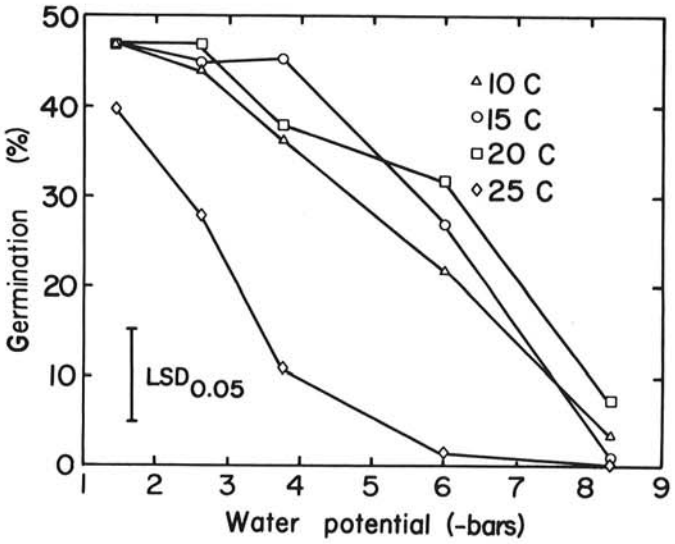

Fig. 5. Germination of teliospores of *Tilletia indica* incubated at 10, 15, 20, or 25 C for 14 days on water agar osmotically adjusted with KCl.

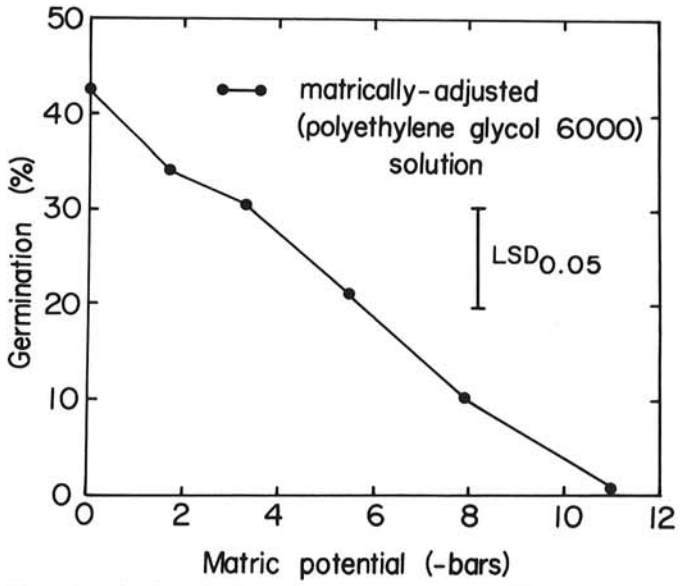

Fig. 6. Germination of teliospores of *Tilletia indica* after 14 days incubation at 15 C on the surface of water solutions matrically adjusted to various water potentials with polyethylene glycol 6000.

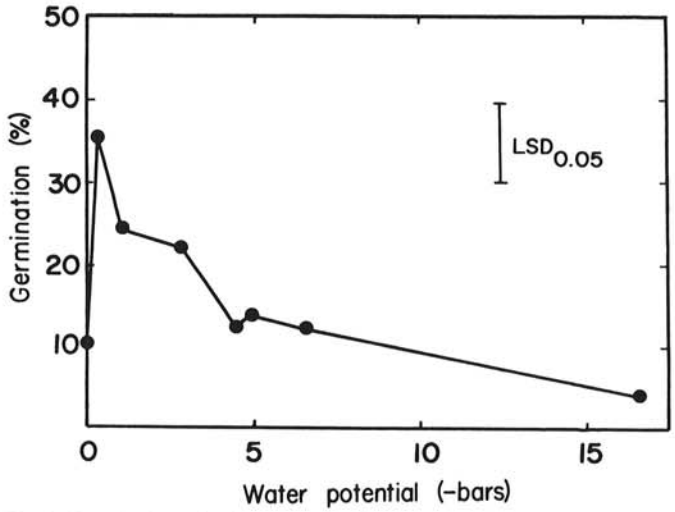

Fig. 7. Germination of teliospores of *Tilletia indica* after 14 days at 15 C on soil adjusted to various matric potentials.

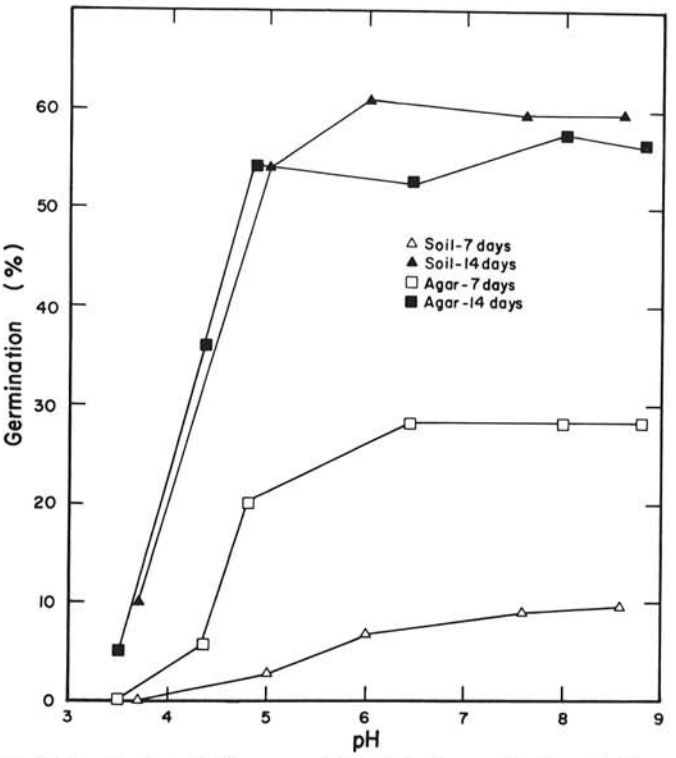

Fig. 8. Germination of teliospores of *Tilletia indica* on pH-adjusted soil or water agar after 7 or 14 days incubation at 15 C.

matric and osmotic media, whereas most fungi are more sensitive to matric potential than osmotic potential (1,15,25). Furthermore, the optimal temperature for teliospore germination did not increase as water potential decreased, as has been reported for the spore germination and growth of many fungi (4,5,11,13,25).

Sufficient knowledge has been obtained to make tentative conclusions regarding the contribution of teliospore germination to Karnal bunt distribution and the disease cycle. Teliospore germination occurs in response to moisture and temperatures from 5 to 25 C and is relatively insensitive to the pH or nutritional composition of the substrate (6,24,33,34). Germination ceases during unfavorable periods of temperature or moisture but resumes unhindered when conditions are again favorable (33). Teliospores remain viable at least 4 yr in soil (23), although their number and viability declines rapidly in warm, moist soil (Smilanick, *unpublished*). Teliospores can survive periods of freezing and thawing; only incubation in frozen (−18 C) soil with a high water content for longer than 2 mo dramatically reduces viability (34). Karnal bunt development is encouraged by poor drainage or excessive irrigation, heavy soils, several days of rainfall, and air temperatures of 15–22 C (3,7,23,27,32). Because these cultural and meteorological factors are conducive to teliospore germination, we suggest that teliospore germination may be a critical determinant in the distribution and severity of this disease. The requirement of relatively high soil moisture for teliospore germination and that teliospores must reside on or very near the soil surface to release infective sporidia (33) suggest that reduced irrigation during the brief period of wheat susceptibility (spike emergence to anthesis) would prevent or greatly reduce teliospore germination and subsequent infection. We have found nothing related to teliospore germination that would suggest a restricted geographic range for this organism. However, many other facets of the epidemiology of Karnal bunt have yet to be elucidated.

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