

EPPO Datasheet: *Xanthomonas translucens* pv. *translucens*

Last updated: 2024-02-28

IDENTITY

Preferred name: *Xanthomonas translucens* pv. *translucens*

Authority: (Jones et al.) Vauterin et al.

Taxonomic position: Bacteria: Proteobacteria:

Gammaproteobacteria: Lysobacterales: Lysobacteraceae

Other scientific names: *Xanthomonas campestris* pv. *hordei* (Hagborg) Dye, *Xanthomonas campestris* pv. *translucens* (Jones et al.) Dye, *Xanthomonas translucens* pv. *hordei* (Hagborg) Dye

Common names: bLS, bacterial leaf streak of barley, bacterial leaf streak of wheat, black chaff of cereals

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EPPO Categorization: A2 list

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EPPO Code: XANTTR



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Notes on taxonomy and nomenclature

The taxonomy of variants belonging to the *Xanthomonas translucens* species has been a topic of confusion and debate in the scientific literature, primarily due to the pest's complex host range infecting many plants within the family Poaceae. This host specificity has contributed to the challenges in accurately classifying and defining this bacterial species. *X. translucens* exhibits a wide range of strains and variants that infect various cereals and grass species. The overlapping symptoms and pathogenicity observed among different strains infecting these diverse hosts have made it difficult to establish clear taxonomic boundaries. The wide variation in host range has long posed significant question regarding whether these strains represent distinct species or simply different pathovars or subspecies within *X. translucens*. According to studies utilizing DNA-DNA hybridization, protein electrophoresis, and fatty acid analysis, *Xanthomonas* strains that were previously known as *Xanthomonas campestris* pathovars of the 'translucens group' infecting Poaceae were reclassified and grouped within the species *Xanthomonas translucens* (Vauterin *et al.*, 1992, 1995). These molecular techniques allowed for a more accurate classification and understanding of the relationships between these strains, resulting in the consolidation of the Poaceae-infecting *Xanthomonas* strains under the species *Xanthomonas translucens*. *X. translucens* pv. *translucens* was classified as strains that caused symptoms when inoculated on barley, but not on other cereals, and includes strains that were previously classified as pv. *hordei* (Bragard *et al.*, 1995, 1997; Sapkota *et al.*, 2020). Recent advancements in genome sequencing have provided valuable insights into the taxonomy of the *X. translucens* species. Through comprehensive comparative genomics analysis of whole-genome sequences, researchers have observed that *X. translucens* can be divided into three distinct clades based on average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) (Goettelmann *et al.*, 2021; Tambong *et al.*, 2023). Pathovar *translucens* belongs to the clade Xt-I along with the closely related pathovar *undulosa*. *X. translucens* pv. *translucens* is further divided into three main subgroups which are globally distributed (Heiden *et al.*, 2022).

HOSTS

While the main hosts of *X. translucens* pv. *translucens* are domesticated and wild barley (*Hordeum vulgare*, *Hordeum marinum*), occasional asymptomatic isolation from wheat (*Triticum aestivum*) and rye (*Secale cereale*) have been recently reported (Curland *et al.*, 2018).

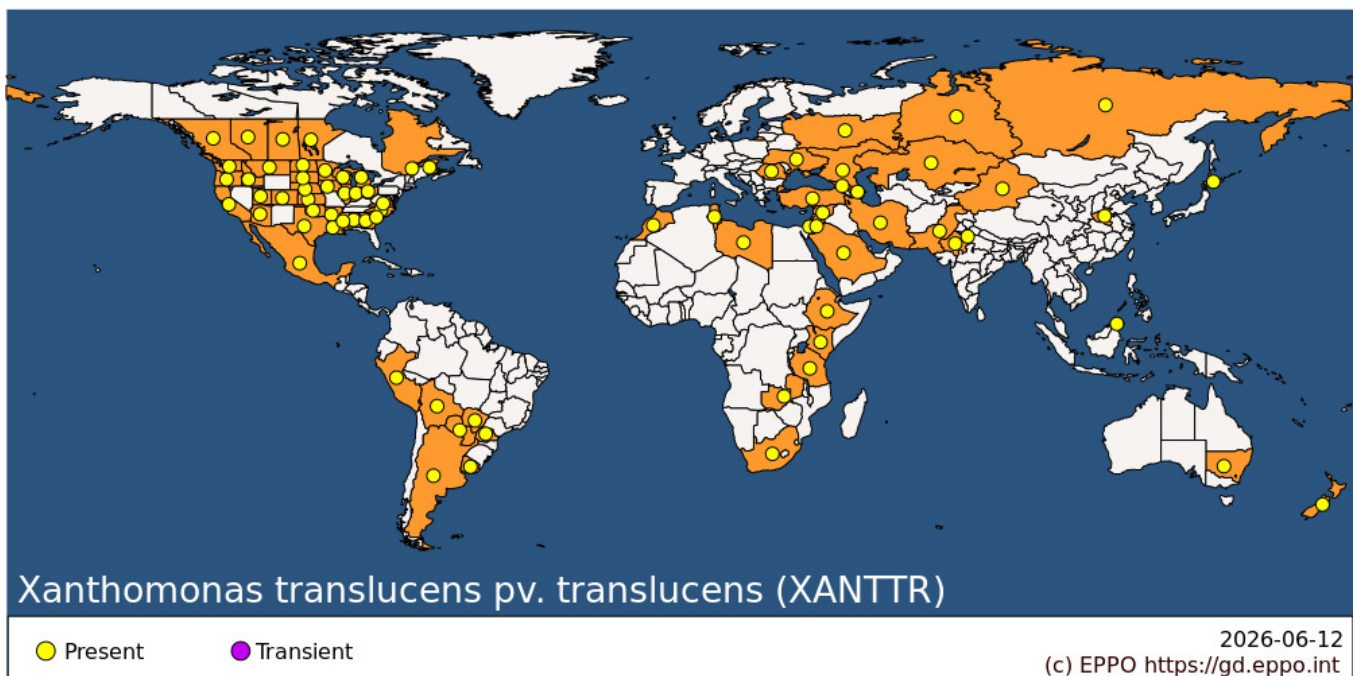
Host list: *Hordeum vulgare*, *Secale cereale*, *Triticum aestivum* subsp. *aestivum*

GEOGRAPHICAL DISTRIBUTION

Bacterial Leaf Blight caused by *X. translucens* pv. *translucens* was first reported near Madison (Wisconsin, USA) in 1912. The accurate determination of the geographical distribution of *X. translucens* pv. *translucens* is challenging due to historical variations in its nomenclature and the misidentification with closely related *X. translucens* pv. *undulosa*. The use of different names for this pathogen in the past has led to confusion and inconsistency in the literature, making it difficult to establish a completely accurate and up-to-date understanding of its geographic range. The similarity in characteristics and symptoms between *X. translucens* pv. *translucens* and *X. translucens* pv. *undulosa* has further complicated the accurate identification and differentiation of these pathogens.

The records given here derive, among other sources, from Bradbury (1986), Duveiller (1989), Duveiller (1994) and are representative of the species *X. translucens*. Based on the available information, it is believed that *X. translucens* pv. *translucens* is present in most barley-growing regions worldwide, with the exception of Western Europe. However, the exact reasons for its absence in Western European barley fields remain unknown. There could be several factors contributing to the absence of the pathogen in Western Europe, but without specific research or studies addressing this issue, it is challenging to determine the exact cause. Some potential factors that could play a role include variations in climate, agricultural practices, or the presence of natural barriers that limit its establishment and spread in the region.

It is noteworthy to mention that recent observations suggest a reemergence of *X. translucens* pv. *translucens* within the barley-growing regions of North America (Curland *et al.*, 2018, 2020; Heiden *et al.*, 2022; Beutler *et al.*, 2023; Ritzinger *et al.*, 2023; Tambong *et al.*, 2023; Schachterle *et al.*, n.d.). This resurgence has raised concerns among researchers and farmers, as it highlights the dynamic nature of pathogen populations and their potential to regain prominence in agricultural systems. Efforts are underway to monitor and study the factors contributing to this reemergence, including shifts in environmental conditions, genetic changes in the pathogen, or alterations in host susceptibility. Analysis of diversity parameters such as haplotype frequency, haplotype diversity, and percentage of polymorphic sites revealed significantly higher genetic diversity in Iranian strains compared to those isolated in North American countries (Khojasteh *et al.*, 2019). Moreover, a global-scale phylogeographic analysis supported the hypothesis that Iranian strains of *X. translucens* have acted as the founding population in other countries, inferring that the centre of origin of the pathogen is from the Middle East and was disseminated in other continents.



- EPP0 Region:** Azerbaijan, Georgia, Israel, Jordan, Kazakhstan, Morocco, Romania, Russian Federation (Central Russia, Eastern Siberia, Southern Russia, Western Siberia), Tunisia, Türkiye, Ukraine
- Africa:** Ethiopia, Kenya, Libya, Morocco, South Africa, Tanzania, United Republic of, Tunisia, Zambia
- Asia:** China (Henan, Xinjiang), India (Delhi, Rajasthan), Iran, Islamic Republic of, Israel, Japan (Hokkaido), Jordan, Kazakhstan, Malaysia (Sabah), Pakistan, Saudi Arabia, Syrian Arab Republic
- North America:** Canada (Alberta, British Columbia, Manitoba, New Brunswick, Québec, Saskatchewan), Mexico,

United States of America (Alabama, Arizona, Arkansas, California, Colorado, Georgia, Idaho, Illinois, Indiana, Iowa, Kansas, Louisiana, Michigan, Minnesota, Mississippi, Montana, Nebraska, North Carolina, North Dakota, Ohio, Oklahoma, Oregon, South Carolina, South Dakota, Texas, Utah, Virginia, Washington, Wisconsin)

South America: Argentina, Bolivia, Brazil (Mato Grosso do Sul, Parana), Paraguay, Peru, Uruguay

Oceania: Australia (New South Wales), New Zealand

BIOLOGY

Seeds have been reported as the primary source of *X. translucens* pv. *translucens* inoculum and large-scale long-distance dissemination of the pathogen (Jones *et al.*, 1916; Jones and Johnson, 1917; Sands *et al.*, 1986; Duveiller *et al.*, 1997). On a local scale, bacteria are transmitted by rain splash, dew and contact between plants (Boosalis, 1951). *X. translucens* pv. *translucens* typically enters the host tissue through existing wounds or through natural water pores called hydathodes. These hydathodes are located at the leaf tips and serve as natural openings for the movement of water and dissolved substances in plants. Hydathodes offer a direct route to the water-transporting xylem vessels where bacteria proliferate at high density and cause systemic disease. While the xylem is the main niche of *X. translucens* pv. *translucens*, at late stages of infection, bacteria eventually exit the xylem vessels and colonize the intercellular space of the mesophyll followed by the release of bacterial exudate at the leaf surface (Shekhawat and Patel, 1978; Gluck-Thaler *et al.*, 2020). The route from which these exudates are released is currently unclear, but stomata may play an important role in this process. One infected plant can lead to an area of 30 m² being infected during a growing season (Sands *et al.*, 1986). It has been hypothesized that aphids and other insects that come into contact with these sticky exudates may carry the bacterium and potentially transmit it to nearby plants, aiding in its dissemination (Boosalis, 1952). However, this has not been demonstrated conclusively and remains largely speculative.

The pathogen exhibits remarkable resistance to dry conditions. The organism has been found to survive for up to eight months on straw obtained from blighted plants and can persist for as long as two years within seeds (Jones and Johnson, 1917). It is worth noting that the survival of *X. translucens* in the field is not solely reliant on the presence of host plants (or host crop residues), as epiphytic populations have been found to persist on non-host species (Timmer, 1987). In the growing season, the optimal temperature range for infection and symptom development is typically between 15 and 22°C (Kim, 1982). The occurrence of disease outbreaks is more common during wet seasons, indicating that warm and humid conditions are critical for the development of the disease (Sapkota *et al.*, 2020). It has been observed that the disease is more prevalent in fields with sprinkler irrigation or during periods of increased rainfall. Inoculation experiments have shown that plants are most susceptible to infection when exposed to moisture, particularly during rainfall or sprinkler irrigation (Sands *et al.*, 1986). It is believed that the presence of moisture on the plant surfaces creates a favourable environment for the bacterium to multiply and establish infections. The wet conditions not only facilitate the survival and multiplication of *X. translucens* pv. *translucens* but may also enhance the likelihood of successful pathogen entry and colonization of the host plant. Preliminary analyses of data from previous field trials seems to indicate that the stage of maturity in barley can significantly influence its response to *X. translucens* pv. *translucens*.

It has been observed that many barley accessions may exhibit apparent levels of resistance to the pathogen before heading, but become more susceptible to infection once they reach the heading stage (Ritzinger *et al.*, 2023). It has been reported that *X. translucens* pv. *translucens* exhibits ice-nucleating activity, which suggests a potential association between the bacterium and frost injury in plants (Kim, 1987; Sands and Fourrest, 1989; Zhao and Orser, 1990). In addition to its potential association with frost injury, the ice-nucleating activity of *X. translucens* pv. *translucens* may also play a role in its dissemination (plants are more easily injured and bacteria may be released from these plants and spread e.g. via rain splash).

DETECTION AND IDENTIFICATION

Symptoms

In the early stages of infection, leaves display distinct symptoms characterized by water-soaked stripe lesions that are prominently visible along the leaf margins when observed under direct light. These lesions appear as areas of abnormal moisture accumulation, giving the affected tissue a soaked and translucent appearance. As the infection

progresses, the water-soaked lesions tend to expand in size and merge, forming larger, streak-shaped lesions. Over time, these lesions can undergo necrosis or tissue death. As the disease advances systemically, blight symptoms may emerge on the infected leaves. Blight symptoms often manifest as wilting, yellowing, or browning of the affected tissue, leading to the overall decline of the leaf. Eventually, the entire leaf becomes desiccated. Downstream of the blight, translucent water-soaked lesions can be found within the mesophyll tissue as a result of local infection. The presence of bacterial slime can be observed at the leaf surface, which subsequently dries and forms a thin scale-like layer that can be flaked off. Symptoms in seedlings may be minimal, while severe infections can cause 'black chaff' symptoms in glumes and seeds, characterized by purple-black discoloration of the surface. It typically takes 10-14 days for symptoms to become apparent. The development of water-soaked symptoms followed by tissue death, as well as the subsequent appearance of blight, are typical patterns associated with *X. translucens* pv. *translucens* infections. These symptoms serve as important diagnostic indicators for this pathogen.

Morphology

X. translucens pv. *translucens* bacteria are gram-negative, non-sporing, rod-shaped aerobic, motile, $0.5\text{--}0.8 \times 1.0\text{--}2.5 \mu\text{m}$ in size, containing a single polar flagellum, and forming typical Xanthomonads yellow colonies on nutrient agar medium (Jones and Johnson, 1917). On Wilbrink's medium, colonies are round, bright, mucoid and yellow (Sands *et al.*, 1986).

Detection and inspection methods

Ideal temperatures for the growth of *X. translucens* pv. *translucens*, similar to many other Xanthomonads, are generally around 28°C. Various non-selective media can be utilized to culture the bacterium, such as Nutrient Agar (NA), Wilbrink's medium, Peptone Sucrose Agar (PSA), and King's Broth (KB) (Jones and Johnson, 1917; Sands *et al.*, 1986). However, for isolation, semi-selective media, including modified Wilbrink's medium (seed and leaf tissue) and mTMB (leaf tissue), are preferred (McGuire, 1986; Sands *et al.*, 1986; Duveiller, 1990). Accurately diagnosing the specific pathogens within the *X. translucens* complex has proven to be a formidable challenge, primarily due to the historical lack of clear taxonomic boundaries surrounding this group and the overlapping host ranges exhibited by these subspecies. As a result, the development of highly specific pathovar-specific diagnostic tools has been lacking. Until recently, detection methods for *X. translucens* included immunofluorescence microscopy, dot-immunobinding assays, and semi-selective enrichment combined with ELISA (Duveiller, 1992; Frommel and Pazos, 1994). However, these methods are not specific to the pv. *translucens* (Bragard and Verhoyen, 1993). This lack of specificity is even more critical due to the fact both *X. translucens* pv. *translucens* and *X. translucens* pv. *undulosa* infect the same host, barley. Inaccurate differentiation between these pathovars can lead to misdiagnosis and the implementation of inappropriate quarantine measure.

PCR tests have recently been developed for *X. translucens* pv. *translucens* which have a high sensitivity and can reliably differentiate it from closely related subspecies within the *translucens* species. A LAMP-based approach has also been developed as a rapid detection and diagnostic method for *X. translucens* in plant tissues (Langlois *et al.*, 2017). This LAMP assay offers the ability to distinguish between clade *Xt*-I, *Xt* clade-II, and *Xt* clade-III within the *X. translucens* species (Goettelmann *et al.*, 2021). However, it does not provide specific differentiation between *X. translucens* pv. *translucens* and *X. translucens* pv. *undulosa* which are both barley pathogens (*Xt*-1 clade). A probe-based real-time PCR protocol was developed for the detection and quantification of *X. translucens* pv. *undulosa*, *X. translucens* pv. *translucens*, and *X. translucens* pv. *secalis* (*Xt*-1 clade) (Sarkes *et al.*, 2022). This real-time PCR protocol provides a reliable and sensitive method for detecting the presence of these pathovars; however, it does not differentiate between the three pathovars. In 2022, a rapid multiplex PCR test was developed to detect all subgroups of *X. translucens* (*Xt*-I to *Xt*-III) and differentiate between *X. translucens* pv. *translucens* and *X. translucens* pv. *undulosa* (Roman-Reyna *et al.*, 2022). Recently, a pathovar-specific TaqMan real-time PCR test was developed with high specificity and analytical sensitivity (Tambong *et al.*, 2023). This test allowed for the specific detection of *X. translucens* pv. *translucens* in plant leaf tissue, with the capability to detect as low as 23 CFU mL^{-1} .

Having a reliable and established seed-detection diagnostic method for this seed-borne pathogen is of utmost importance. To ensure the safe trade of agricultural commodities, robust diagnostic tools are needed. Therefore, it is important to highlight that the LAMP assay developed by Langlois *et al.* (2017) has undergone testing and validation for the detection of the pathogen in barley seeds. In contrast, the study conducted by Sarkes *et al.* (2022) focused on the detection of *X. translucens* pv. *undulosa* specifically in wheat seeds. Even though these approaches do not offer specific differentiation between barley-infecting *X. translucens* pv. *translucens* and *X. translucens* pv. *undulosa*,

their capability to detect both pathogens on seeds makes them valuable and practical seed-detection methods.

PATHWAYS FOR MOVEMENT

The bacteria are primarily dispersed locally through splashing over short distances. However, the potential for international spread is primarily associated with infected seed lots.

It is crucial to highlight that the presence of a conserved ice-nucleation gene among *X. translucens* pv. *translucens* strains indicates its capability for aerial dispersal and long-distance dissemination. Additionally, the isolation of *X. translucens* from rain events further supports this notion (Failor *et al.*, 2017).

PEST SIGNIFICANCE

Economic impact

Over the last two decades, *X. translucens* pv. *translucens* has re-emerged and raised significant concerns in barley production (Sapkota *et al.*, 2020). Infections caused by this pathogen can have severe adverse effects on both crop yield and quality (Sapkota *et al.*, 2020; Ritzinger *et al.*, 2023). However, there is limited quantitative data regarding the extent of losses caused by this pathogen. This can be attributed to two main factors: the absence of completely resistant barley varieties for comparative analysis and the lack of studies specifically targeting the impacts of *X. translucens* pv. *translucens* on barley. Consequently, the understanding of the precise economic and agronomic consequences of *X. translucens* pv. *translucens* infections remains relatively limited. Further research and investigations are necessary to fill this knowledge gap.

Control

A combination of management strategies is advisable to control Bacterial Leaf Blight on barley. These include implementing a seed certification program, to ensure the absence of the bacterium in seeds (Forster and Schaad, 1988; Duveiller *et al.*, 1997). Efficient screening and monitoring of seed lots during production, storage, and distribution processes are essential. While there are currently no seed treatments available that provide complete effectiveness against the pathogen, it is possible to implement quarantine measures to prevent the unintended introduction of the pathogen into new regions (Duveiller *et al.*, 1997).

Additionally, the following agricultural practices can be used:

Cultural control

Implementing a crop rotation scheme that includes nonhost plants can help break the disease cycle and reduce the viable inoculum present in the field (Ritzinger, 2022). Furthermore, removing infected crop residue after harvest may help in preventing the survival and spread of the pathogen (Ritzinger *et al.*, 2023). It is also advisable to eliminate potential alternative host weeds located in close proximity to barley fields, as the closely related pathovar *X. translucens* pv. *undulosa* was detected on a multitude of weedy grasses typically found around wheat field (Ledman *et al.*, 2021).

Plant resistance

Due to the lack of effective chemical control, host resistance is currently considered to be the most efficient disease management strategy for *X. translucens* pv. *translucens*. Consequently, prioritizing germplasm screening for resistance to bacterial leaf blight becomes crucial in management strategies. Previous studies aimed to develop more resilient barley varieties. The resistant cultivar Oderbrucker which was identified as early as 1917 by Jones *et al.* exhibited resistance in field trials in Montana (Kim, 1982). Three quantitative trait loci (QTLs) associated with resistance to Bacterial Leaf Blight were identified on chromosomes 3 (two QTLs) and 7 (one QTL) of the cultivar Morex, a widely deployed six-row spring malting barley that was released in 1978 by the Minnesota Agricultural Experiment Station (Alizadeh *et al.*, 1994). This cultivar has gained popularity in North American barley fields and serves as the current reference genome for barley. The QTL located near the marker ABG377 on chromosome 3 was

found to be the primary contributor to the resistance. However, this resistance is only partial and Morex remains susceptible to the current *X. translucens* pv. *translucens* population in North America (Jules Butchacas, personal communication). In a recent study, a panel of 2094 barley accessions was subjected to field trials conducted in Minnesota (Ritzinger *et al.*, 2023). By considering both resistance during leaf development and heading time, the authors were able to narrow down the list of potential Bacterial Leaf Blight-resistant candidates for breeding to 32 accessions. The study also revealed that wild barley parents from countries such as Israel, Lebanon, Iran, Turkmenistan, Azerbaijan, Syria, Türkiye, and Jordan played a significant role in contributing Bacterial Leaf Blight resistance alleles. Wild barley species (e.g. *H. spontaneum*, *H. marinum*) have been widely acknowledged for their valuable resistance against various fungal pathogens affecting barley, confirming their potential as a valuable resource for breeding and developing barley varieties with resistance to Bacterial Leaf Blight (Kim, 1982; Dinh *et al.*, 2022). Ritzinger *et al.*, 2023 once again demonstrated the resistance of 'Oderbrucker' to Bacterial Leaf Blight. Ritzinger *et al.* (2023) underlined the importance of developing genetic markers through genome-wide association studies (GWAS) and classical mapping studies for breeding programs aimed at enhancing Bacterial Leaf Blight resistance in barley. These genetic markers provide essential insights into the underlying genetic mechanisms of Bacterial Leaf Blight resistance and enable targeted breeding efforts.

It is important to acknowledge that the identified germplasm sources resistant to Bacterial Leaf Blight present poor agronomic characteristics. Research should continue to develop varieties that combine resistance to Bacterial Leaf Blight with favourable agronomic characteristics to provide farmers with improved varieties that are resistant to the disease and also possess desirable traits such as high yield potential, good quality, and adaptability to different environmental conditions.

Phytosanitary risk

X. translucens pv. *translucens* can have negative impacts on the yield of barley, an important crop in the EPPO region. While it is most likely already present within the EPPO region, accurately establishing its exact range poses challenges due to symptom confusion and the historical lack of reliable detection methods. Given its seed-borne nature and its presence in areas with similar climates as in the EPPO region, there is a potential risk for *X. translucens* pv. *translucens* to further establish and develop in new parts of the EPPO region if infected seeds are used.

PHYTOSANITARY MEASURES

To minimize the risk of introducing *X. translucens* pv. *translucens*, it is essential to adopt stringent measures for import and exchange of barley seeds. Therefore, robust diagnostic tests and rigorous phytosanitary measures are essential in preventing the unintentional introduction and spread of this pathogen to new areas within the EPPO region. Seeds of host plants should either come from a pest-free area or be certified as pest free if they are produced in countries where the pest occurs. Such measures should also apply to barley seeds imported for breeding purposes in areas where the pest does not occur. To mitigate this risk, it is advisable to produce such seeds in dry and disease-free zones whenever possible, supplemented by regular testing.

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