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*SHORT NOTES*

# Response of commonly cultivated tomato cultivars in Nepal to bacterial speck

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**Summary.** Ten commonly cultivated cultivars of tomato (*Solanum lycopersicum* L.) in Nepal were tested in a plastic tunnel and in the field for their susceptibility to *Pseudomonas syringae* pv. *tomato*, the causal agent of bacterial speck. Four of the ten cultivars were local and six were hybrids. The leaves of each cultivar were sprayed with *P. s.* pv. *tomato* both in the tunnel and in the field. The genotypes exhibited a considerable variation in response to infection, with the disease severity index (DSI) varying from 1.80 to 4.25 in the field and from 1.10 to 4.20 in the tunnel. The cultivars Thims 16, C.L. and Spectra 737 were the least susceptible in the field, with DSI values of 1.80, 2.05 and 2.25, respectively; while in the tunnel all the local cultivars (C.L., Panjabi, B.L. and Lapsi Gede) showed very low susceptibility, with respective DSI values of 1.10, 1.20, 1.65 and 2.30. In the field, the most susceptible cultivar was Lapsi Gede (DSI=4.25) and in the tunnel the most susceptible was NS-719 (DSI= 4.20).

**Key words**: cultivar susceptibility, *Solanum lycopersicum*, *Pseudomonas syringae* pv. *tomato*.

## Introduction

Tomato is one of the most important vegetable crops grown in Nepal (Ghimire *et al*., 2000) with more than 10.000 ha under tomato cultivation and an average yield of 72.000 tonnes (Shrestha and Ghimire, 1996). Because of variations in the climate within the country, tomatoes are grown in winter, in spring and in the rainy season in the south (Terai and inner-Terai) while it can be grown in two seasons (spring and summer) in the low and mid hills. In the hills tomatoes used to be grown only in the summer at subsistence level, but recently the availability of hybrids on the market has made it possible to cultivate the crop in spring as well.

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An important disease in many tomato-growing areas of the world is bacterial speck of tomato caused by *Pseudomonas syringae* pv. *tomato* (Okabe) Young, Dye and Wilkie (Pst). The Pst pathogen has two races, race 0 and race 1 (Habazar and Rudolph, 1997). Race 0 is the most widespread in countries where tomato is grown (Goode and Sasser, 1980; Varvaro and Guario, 1983) and it has recently also been reported from Nepal (Lamichhane *et al*., 2009); while race 1 is less widespread but occurs in several countries, including Canada and Italy (Lawton and MacNeill, 1986; Buonaurio *et al*, 1996). The disease causes serious economic losses especially on fruits of susceptible genotypes (Zaccardelli *et al*., 2003). The pathogen possesses an extraordinary ability to survive epiphytically on weeds and even on symptomless tomato transplants, as well as in the soil and in seed (Devash *et al*., 1980; McCarter *et al*., 1983). All these characteristics make the disease diffi-

cult to control by cultural practices and spraying with copper preparations, although some positive results have recently been obtained with natural extracts (Jones and Jones, 1989; Ramos *et al*. 1989; Da Silva *et al*. 1995; Varvaro *et al*. 2001; Wilson *et al*. 2002; Balestra *et al*. 2009). Planting disease-free material is essential for cost-effective yield since prevention is the key to controlling bacterial speck of tomato (Goode and Sasser, 1980). Planting tomato cultivars resistant or less susceptible to race 0, which is the most widespread, may be the best control strategy, which is generally easy to use and economic. Additionally, it avoids the severe losses in yield (Yunis *et al*., 1980; Volin and Pohronezny, 1983). The aim of the present study was to determine the susceptibility to bacterial speck of ten tomato cultivars commonly grown in Nepal.

## Material and methods

Experiments were carried out at the Central Horticulture Center, Kirtipur, in Kathmandu District. Experiments in the shaded plastic tunnel and in the field were both conducted in the spring of 2009.

### Tomato plants

Four local cultivars (B.L., C.L., Panjabi and Lapsi Gede) and six Asian hybrids of various origin (Srijana, Thims 16, Manisha, NS-719, Bishesh and Spectra 737), commonly grown in Nepal, were tested. 'Srijana' and 'Thims 16' were Nepalese hybrids, 'Manisha' and 'NS-719' were Indians, 'Bishesh' was Thai and 'Spectra 737' was a hybrid from South Korea. The seeds were sown directly in a seedbed prepared with sand and soil in a ratio of 1:1. Before sowing, the seeds were pre-germinated for 36 hours in a Petri dish lined with moistened paper.

### Preparation of plastic pots and transplanting

Two weeks after sowing, the tomato seedlings were transplanted. For the tunnel experiment, they were transplanted into 1.5 litre plastic pots (10 cm wide and 25 cm deep) containing soil and sand (1:1) and kept in the tunnel. For the field experiment, the seedlings were transplanted directly to the field. In either experiment twenty plantlets per cultivar were tested.

### Bacterial strains and preparation of bacterial suspension

Strain PST 5N07 race 0, was used for the susceptibility test. This strain has been previously isolated in Nepal (Lamichhane *et al*., 2009) and deposited at GenBank (Accession FJ590508).

The bacterial isolate, preserved on nutrient agar (NA) supplemented with 2% of glycerol (NGA), was streaked on NA. After 24 hours, the fresh bacterial culture was re-streaked on the same medium, forming a dense bacterial culture. To prepare the bacterial suspension, the bacterial culture was added to a beaker containing sterilized distilled water (SDW), producing a suspension that was centrifuged at 15,000 × *g* for 20 min. The pellet was used to obtain a homogeneous bacterial suspension in SDW. The concentration of the bacterial suspension was adjusted turbidimetrically to about $10^8$ colony forming unit (CFU) $mL^{-1}$ by reference to a calibration curve (Varvaro and Surico, 1987).

### Inoculation of plants

The leaves of 3–4-week-old tomato plants, consisting of ten plants per cultivar in the field and the same number in the tunnel, were sprayed homogeneously with the bacterial suspension (Santangelo *et al*., 1998). Two control plants per cultivar in the field and an equal number in the tunnel, sprayed only with SDW, were used as the control. All the plants were covered with plastic bags from 2 hours before until 2 hours after spraying to maintain a high relative humidity (90 to 100%). Plants in the field and those in the tunnel were inoculated on the same day. In both the field and the tunnel the humidity varied from 50 to 70%. The mean temperatures were 30±3°C by day and 22±3°C at night in the field, and 25±3°C by day and 20±3°C at night in the tunnel.

### Symptoms evaluation

Symptoms were inspected 2, 4, 6, 8 and 10 days after inoculation, counting the number of visible speck lesions on the whole leaves of all plants. The average number of lesions on each compound leaf was calculated by dividing the total number of lesions by the total number of compound leaves. The overall rating for each cultivar was calculated using the scale of Chambers and Merriman (1975), slightly modified as follows: 0, no lesions; 1, 1–15

lesions per leaf; 2, 16–30 lesions per leaf; 3, 31–60 lesions per leaf; 4, 61–120 lesions per leaf and 5, more than 120 lesions per leaf (Figure 1). Bacterial speck was evaluated on the basis of the disease severity index (DSI).

**Measurement of lesion size**

Ten days after the inoculation, two leaves per plant per cultivar were randomly collected. The diameters of any necrotic lesions were measured using an optical microscope at a magnification of 2.5×. The average lesion diameters were calculated for each cultivar.

**Statistical analysis**

Data from each experiment for both years were combined, averaged and subjected to analysis of variance (ANOVA). Duncan's multiple range test was used to calculate the differences within and between cultivars.

## Results

**Symptoms on tomato plants**

Foliar lesions caused by Pst were visible from 2 to 4 days after inoculation both in the field and in the tunnel. In the field experiments, all the hybrid cultivars showed symptoms within 2 days of inoculation except the cv. Bishesh which took four days; whereas on the local cultivars, the symptoms did not appear until 4 days, except for cv. Lapsi Gede, which had them after two days. The only difference between the field and the tunnel experiments was with cv. Lapsi Gede, on which symptoms in the tunnel appeared after 4 days instead of 2 days in the field. Both field and tunnel grown plants had the same symptoms. No symptom appeared on the control plants.

**Disease evaluation**

*Disease severity index*

No tomato cultivar artificially inoculated with Pst was totally resistant to the pathogen. However, cultivars varied in susceptibility (Table 1). At the end of the experiment, 10 days after inoculation, the DSI ranged from 1.80 to 4.25 in the field. The hybrid 'Thims 16' was the least susceptible (DSI=1.80) together with the local cvs. C.L. (DSI=2.05) and Spectra 737 (DSI=2.25). The local cv. Lapsi Gede was the

Table 1. Disease severity index (DSI) in the field and in a tunnel for tomato cultivars 10 days after inoculation with *Pseudomonas syringae* pv. *tomato* (PST 5N07).

| Cultivar | DSI in the field ± SE | | | DSI in the tunnel ± SE | | |
|---|---|---|---|---|---|---|
| Thims 16 | 1.80 | bc[a] | ±0.20 | 3.45 | fghi[a] | ±0.17 |
| C.L. | 2.05 | bcd | ±0.23 | 1.10 | a | ±0.10 |
| Spectra 737 | 2.25 | cd | ±0.19 | 2.90 | ef | ±0.20 |
| Bishesh | 2.60 | de | ±0.20 | 3.05 | efgh | ±0.21 |
| B.L. | 3.00 | efg | ±0.25 | 1.65 | ab | ±0.18 |
| NS-719 | 3.05 | efgh | ±0.18 | 4.20 | jk | ±0.21 |
| Panjabi | 3.55 | ghi | ±0.22 | 1.20 | a | ±0.12 |
| Srijana | 3.60 | ghi | ±0.17 | 3.60 | ghi | ±0.18 |
| Manisha | 3.65 | hij | ±0.17 | 3.75 | ijk | ±0.16 |
| Lapsi Gede | 4.25 | k | ±0.22 | 2.30 | cd | ±0.21 |

[a] Means followed by the same letter are not significantly different at $P$=0.05.

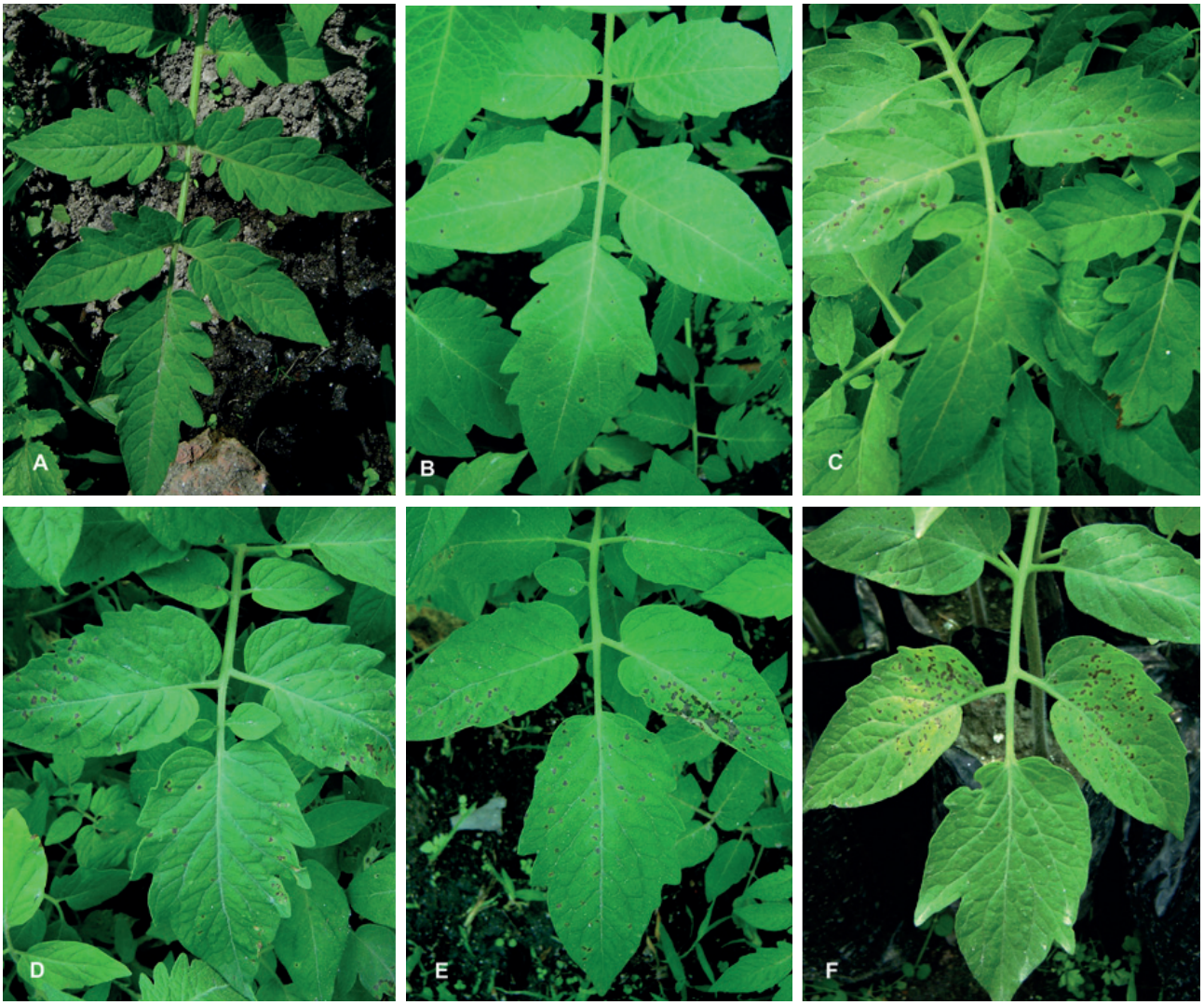


Figure 1. Determination of the Disease Severity Index based on the number of lesions on tomato leaves, using a scale from 0 to 5. A, no lesion. B, 1–15 lesions. C, 16–30 lesions. D, 31–60 lesions. E, 61–120 lesions. F, more than 120 lesions.

most susceptible (DSI=4.25). By contrast, in the tunnel the local cultivars C.L., Panjabi and B.L. were the least susceptible (with a DSI values of 1.10, 1.20 and 1.65 respectively), followed by 'Lapsi Gede' (DSI=2.30). The hybrids were more susceptible with a DSI from 2.90 to 4.20. The cv. NS-719 was the most susceptible (DSI=4.20).

Some cultivars reached their maximum DSI after only few days and this DSI remained constant until the end of the experiments (Figure 2). The local cv. B.L. and C.L. in the field reached their maximum DSI 6 days after inoculation, whereas the cv. Thims 16, NS-719, Panjabi, Spectra 737, Srijana and Manisha did not reach their maximum DSI until 8 days after inoculation in the field and in the tunnel, and the remaining cultivars reached the maximum DSI only after 10 days; these differences were statistically significant. The hybrids Srijana, Bishesh, Spectra 737 and Manisha and the local cv. Lapsi Gede in the field, and the hybrids Thims 16 and all local cultivars in the tunnel did not reach their maximum DSI until 10 days after inoculation.

**Lesion diameter**

Diameters of the lesions formed on the leaf surface of tomato plants, from all cultivars and hybrids, both in the field and in the tunnel, were similar (Table 2). The average diameter ranged from 1.37 to 1.95 mm in the field and from 1.49 to 1.78 mm in the tunnel. Lesion diameters did not differ significantly between susceptible and resistant cultivars.

## Discussion

The time required by the tomato cultivars for symptom appearance did not differ significantly between the field and the tunnel experiments. These symptoms consisted in lesions that were generally surrounded by a yellow chlorotic halo caused by the phytotoxin coronatine (Bender *et al*. 1999) produced by the bacterium and they ap-

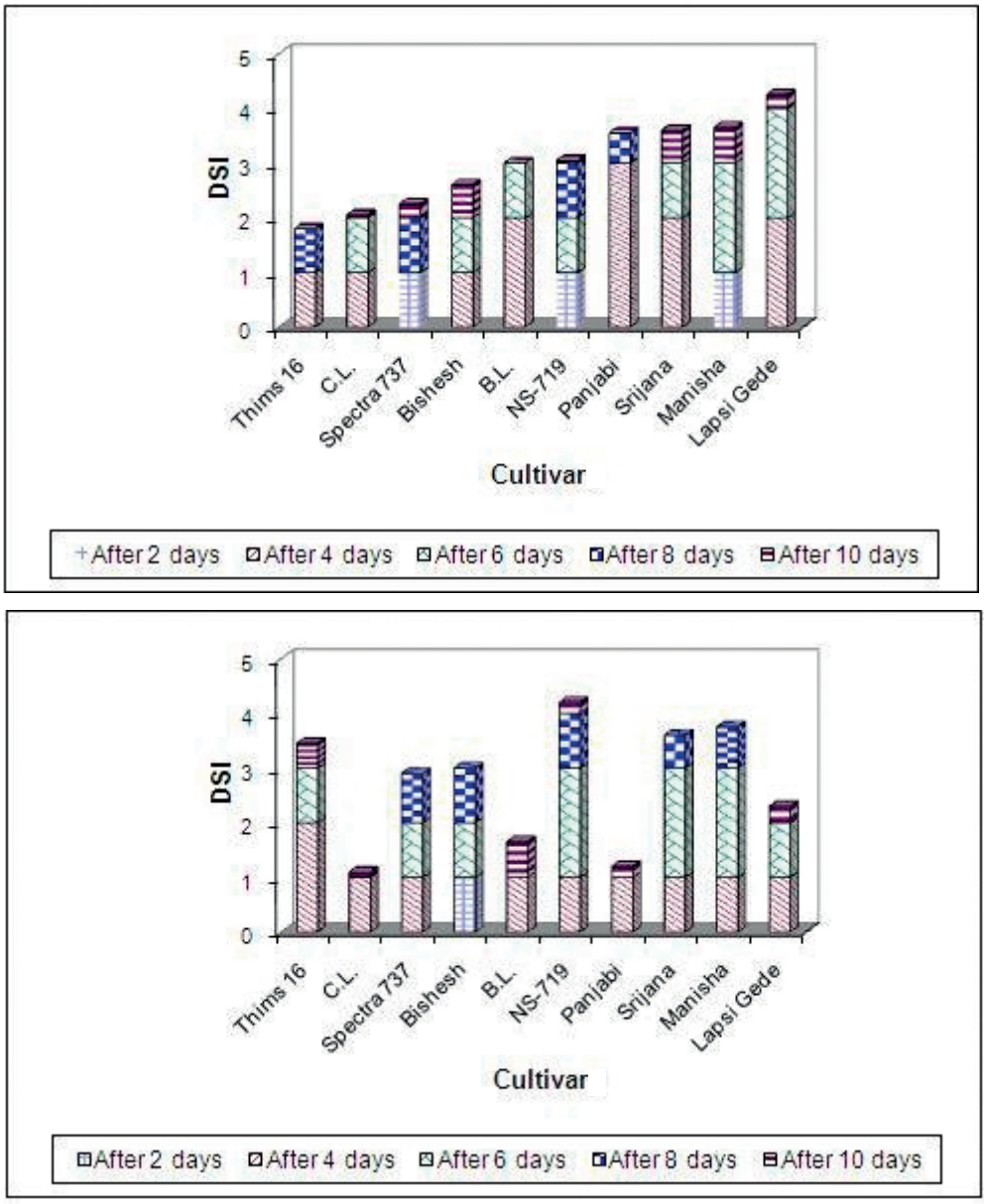


Figure 2. Disease severity index of tomato cultivars 2, 4, 6, 8 and 10 days after inoculation with *Pseudomonas syringae* pv. *tomato* (PST 5N07). A, In the field experiment. B, In the tunnel experiment.

peared at the same time after inoculation in both the field and the tunnel experiments, probably because the higher temperature of the test conditions accompanied by a high relative humidity accelerated the multiplication of bacteria and hence the appearance of symptoms (Smitley and McCarter, 1982).

The DSI did not differ significantly between the hybrids and the local cultivars in the field, where mixed responses were obtained from both the hybrids and the local cultivars, with some being less susceptible, and others more. In the tunnel experiment, on the other hand, there was a clear difference between the DSI hybrids and that of the local cultivars: all local tomato cultivars were less susceptible, with a lower DSI than the hybrids. This is probably due to the fact that the lower temperatures inside the shaded tunnel were favourable for the local cultivars, leading to a lower DSI, since these cultivars have always been cultivated in the cool areas of the mountain slopes. However, the difference in susceptibility between the hybrids and the local cultivars was probably caused by differences in the multiplication rate of Pst on the phylloplane of the less susceptible cultivars (Babelegoto *et al*., 1988).

No correlation existed between the number of lesions on the leaves and the diameter of the lesions since almost all cultivars had lesions with a similar average diameter, even if the DSIs of these lesions differed.

Though the susceptibility of the local cultivars in the tunnel was low (DSI between 1.10 and 1.65) when compared with the Nepalese hybrid Thims 16 and the local cultivar C.L. in the field (DSI between 1.80 and 2.05), Thims 16 and C.L. could be recommended to tomato growers since almost all tomatoes in Nepal are field-grown. Rather than some cultivars having a more susceptible response than others, all cultivars gradually increased their DSI, showing that when climatic conditions are favourable, the pathogen will cause severe economic losses irrespective of the cultivar.

In the Kathmandu valley bacterial speck of tomato was first seen on the cultivar B.L. (Lamichhane *et al*., 2009), a common rainy season tomato cultivar grown in different parts of Nepal. The disease will now be extensively monitored in commercial tomato growing areas of the country to determine its incidence and the yield losses associated with it, and to find out how to control the spread of the disease to other parts of the country where the pathogen is unknown.

Table 2. Average lesion diameters (mm) formed on tomato leaf in field and in a tunnel 10 days after inoculation caused by *Pseudomonas. syringae* pv. *tomato* (PST 5N07).

| Cultivar | Field | | | Tunnel | | |
|---|---|---|---|---|---|---|
| Srijana | 1.37 | a[a] | ±0.26 | 1.55 | ab[a] | ±0.33 |
| Spectra 737 | 1.54 | ab | ±0.36 | 1.50 | ab | ±0.33 |
| NS-719 | 1.61 | abc | ±0.33 | 1.56 | ab | ±0.31 |
| Bishesh | 1.65 | abc | ±0.27 | 1.72 | abc | ±0.27 |
| Lapsi Gede | 1.69 | abc | ±0.31 | 1.49 | ab | ±0.24 |
| Panjabi | 1.69 | abc | ±0.29 | 1.57 | ab | ±0.27 |
| Thims 16 | 1.72 | abc | ±0.27 | 1.78 | abc | ±0.32 |
| C.L. | 1.84 | b | ±0.31 | 1.64 | abc | ±0.27 |
| Manisha | 1.89 | b | ±0.34 | 1.71 | abc | ±0.29 |
| B.L. | 1.95 | c | ±0.34 | 1.54 | ab | ±0.23 |

[a] See Table 1.

Among the factors limiting tomato yield in Nepal, Pst is one of the most serious especially for rainy season tomatoes. Since the pathogen has already been reported, and since the study found that both spring (hybrids in particular) and summer tomatoes (local varieties) are susceptible, it is strongly recommended that appropriate control strategies should be implemented to limit the spread of the pathogen. If the disease is detected, infected plants in the field should be eliminated and the marketing of seeds should be carefully controlled to avoid the spread of infected seeds.

Since the cultivars tested in this study are grown in most tomato-growing areas in Nepal (about 80 %) during the entire tomato growing season, bacterial speck may be expected to cause serious losses in yield if the inoculum concentration become high enough. So far bacterial speck has only been considered a low profile disease in the areas where it was found, but heavy outbreaks can be expected, since the rainy seasons in the different tomato-growing areas of Nepal are characterized by a high relative humidity accompanied by high temperatures, and these are the ideal conditions for the pathogen to infect plant tissues and produce symptoms (Pietrarelli *et al*. 2006).

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