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Research Article

SURVEY AND SURVEILLANCE FOR DISEASE PREVALENCE AND INCIDENCE OF STALK ROT OF MAIZE IN KARNATAKA

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Abstract: Stalk rot disease of maize is a serious problem in all major maize growing regions but the exact disease incidence and estimated loss in Karnataka is not known. Therefore, in the present study, field survey was conducted in major maize growing districts of Karnataka during the three *Kharif* seasons from 2013-15. Rowing field survey was conducted to determine the incidence of stalk rot. A total of 10 maize growing districts were surveyed and the data were collected on the incidence of stalk rot. The study also reported the prevalence of stalk rot in all maize producing regions. The highest percent prevalence of stalk rot disease was recorded in Bellary region (66.66%). The maximum mean incidence was observed in Bellary and Koppal districts (>24% mean incidence) and least was in Dharwad and Haveri (<5% mean incidence). Similarly, maximum disease incidence was recorded in Shankarbande regions of Bellary followed by Halagere (Koppala) with maximum incidence of >40%. The isolation frequency of *Fusarium* species associated with stalk rot diseases ranged from 45 -57%. Study also indicated that, several other fungal pathogens were also associated with the stalk rot. However, incidence of *Fusarium* was dominant in all the study areas. *Fusarium* species isolated from infected stalks were identified based on colony characteristics and morphological features. In conclusion, the present study determined the actual disease incidence and prevalence of stalk rot disease in major maize growing districts of Karnataka.

Keywords: *Disease Incidence, Pathogenicity, Epidemiology, Stalk Rot of Maize*

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Introduction

Maize (*Zea mays* L.) is one of the most important food crop of the world [1] with the global area under maize cultivation is approximately 183 Mha with a production of 1065 MT and productivity of 5.82 tonnes/ha during 2013-14 [2]. United States of America (USA) is the largest maize producer and it is also having a large surplus, which makes it the largest maize exporter. Brazil, Ukraine and Argentina are the other key maize producing countries behind USA and these four countries together account for 80-85% of the total exports in maize. In India, maize is the third most important cereal crop after rice and wheat, accounting for ~9 percent of total food grain production. It is cultivated throughout the year almost in all the states of the country for various purposes. It was planted in an area of 9.06 Mha during 2013-14 with a production of 24.25 MT and productivity of 2.7 T/ha [2]. Karnataka, Andhra Pradesh, Bihar, Madhya Pradesh, Rajasthan, Gujarat, Maharashtra, Tamil Nadu, Uttar Pradesh and Chhattisgarh are the major maize growing states in India. Many fungal and bacterial pathogens are known to attack maize, among these, *Ustilago maydis* causing corn smut, *Exserohilum turcicum* causing leaf blight (or northern corn leaf blight), *Cochliobolus heterostrophus* causing seedling and leaf blight (or Southern corn leaf blight) and *Fusarium* (*Teleomorph Gibberella*) species causing ear rot, stalk rot and seedling blight [3-8] are the major constraints to maize production.Stalk rots are among the most destructive disease in maize throughout the world [9,10]. *Fusarium* stalk rot is one of the most widespread and destructive diseases of maize worldwide. The disease is caused by a complex of several fungal pathogens and secondary colonizers. The important species of *Fusarium*, reported to cause stalk rot from various regions includes *F. moniliforme*, *F. graminearum*, *F. temperatum*, *F. subglutinans* and *F. proliferatum* are associated with maize at global level [11-14]. Several species of *Fusarium* are important due to their ability to cause disease in important crops, and to produce a variety of mycotoxins such as moniliformin, fumonisin, deoxynivalenol (also known as vomitoxin), beauvericin, fusaproliferin and fusaric acid [4,15]. Contaminated grains with high levels of mycotoxins can be fatal and long-term exposure to mycotoxins causes health risks [16, 17]. Maize is an important commercial crop cultivated in all major agro-ecological regions of Karnataka and the area of cultivation is increasing every year. However, no data is available on the incidence of stalk rot disease in Maize and the extent of damage or loss caused due to stalk rot disease of maize. Therefore, the present study was conducted to determine the prevalence, incidence and severity of stalk rot disease in 10 major maize producing districts of Karnataka during *Kharif* season from 2013 to 2015.

Materials & Methods

Study area

The present study was conducted in 10 major maize producing districts of Karnataka state during 2013-15 *kharif* seasons. The districts regions selected in the study are Belgaum, Bagalkot, Davanagere, Haveri, Chitradurga, Bellary, Koppala, Gadag, Dharwad and Chikkaballapur [Fig-1]. The survey area covered approximately 600 fields in each year.

Disease Prevalence

In all the 10-major maize growing districts disease prevalence was estimated during the *kharif* seasons.

Table-1 *Number fields surveyed for three consecutive years (2013-15) for Disease prevalence during Kharif season*

SN	District	Taluks	2013		2014		2015	
			N*	DP** (%)	N	DP	N	DP
1	Belgaum	Bailhongal	26	85	12	25	10	80
		Saundatti	20	60	25	36	26	54
		Gokak	16	88	19	53	14	57
		Ramdurga	10	30	14	36	26	65
		Mean	72	65.75±26.93	70	37.50±11.56	76	64.00±11.63
2	Bagalkot	Badami	18	72	23	74	13	23
		Jamakhandi	15	51	31	52	17	35
		Mudhol	28	52	14	57	22	14
		Mean	61	58.33±11.84	68	61.00±11.53	52	24.00±10.53
3	Davanagere	Davanagere	27	48	22	45	18	28
		Harappanalli	15	35	27	33	20	50
		Jagalur	14	73	14	71	26	54
		Honnalli	10	75	10	70	23	74
		Mean	66	57.75±19.51	73	54.75±18.83	87	51.50±18.85
4	Haveri	Haveri	10	58	12	58	31	52
		Shiggaon	82	52	24	54	14	57
		Savanur	20	70	28	71	22	45
		Ranebennur	30	24	10	20	27	33
		Hirekerur	25	54	19	53	23	74
		Hanagal	31	29	18	28	14	73
		Mean	198	47.83±17.73	111	47.33±19.34	131	55.66±15.99
5	Chitradurga	Chitradurga	14	50	15	53	10	75
		Chellakere	10	70	25	76	10	58
		Mean	24	60.00±14.14	40	64.50±16.26	20	66.50±12.02
6	Bellary	Bellary	20	24	12	25	82	52
		Hagaribommenalli	23	54	16	56	14	73
		Huvinahadagali	42	29	24	33	10	75
		Mean	85	35.66±16.07	52	38.00±16.09	106	66.66±12.74
7	Koppala	Koppala	18	50	35	51	23	74
8	Gadag	Nargund	28	90	16	88	31	52
		Gadag	16	50	13	54	14	57
		Rona	28	70	19	68	22	45
		Mean	90	65.00±20	83	65.25±17.08	90	57.00±6.02
9	Dharwad	Dharwad Taluk	12	24	15	20	14	73
		Hubli Taluk	14	54	22	55	10	75
		Navalgund Taluk	28	29	12	33	23	74
		Kalaghatagi Taluk	22	50	28	50	31	52
		Kundagol Taluk	13	45	14	57	14	57
		Mean	89	40.40±13.20	91	43.00±15.95	92	66.2±10.84
10	Chikkaballapur	Gauribidanur	20	74	22	73	22	45
Mean (Including all ten districts) 32			705	53.71±19.23	610	50.87±17.88	676	56.4±17.02

*Note: Sampling was done during Kharif season; *N-Total number of fields evaluated in that region; DP**-Disease Prevalence was estimated by using each field as one unit (Number of fields with stalk rot incidence to the total number of fields evaluated for stalk rot incidence).*

Table-2 *Mean incidence and highest incidence of stalk rot of maize in major maize growing regions of Karnataka during the Kharif season 2013 – 2015.*

Districts	Mean Severity 2013	Mean Severity 2014	Mean Severity 2015	Locations (with highest severity)	Highest Severity 2013	Highest Severity 2014	Highest Severity 2015
Belgaum	9.93	10.16	8.39	Narsapur (Ramdurg Taluk)	24.58	25.74	23.06
Bagalkot	12.38	12.21	10.92	Muddegannur (Mudhol Taluk)	31.11	30.90	28.96
Davanagere	7.57	7.01	6.17	Keregudalli (Harappanalli Taluk)	16.80	15.14	14.88
Haveri	5.02	4.87	4.60	Devagiri (Haveri)/Sunakalbidar	20.50	18.83	19.63
Chitradurga	8.49	8.66	6.41	Chellakere (Chellakere Taluk)	20.71	21.53	18.59
Bellary	24.90	24.34	24.45	Shankarbande (Bellary Taluk)	42.94	42.23	42.55
Koppala	24.45	24.60	22.60	Halagere (Koppala)	36.15	39.55	37.90
Gadag	9.54	10.60	10.26	Nargund (Nargund)	22.66	23.58	22.94
Dharwad	5.05	4.80	4.52	Dharwad (Dharwad Taluk)	13.02	12.56	12.18
Chikkaballapur	13.93	14.13	12.60	Thondebavi (Gauribidanur Taluk)	29.93	31.15	29.25

A total of 705, 610 and 676 fields were assessed during the year 2013, 2014 and 2015 respectively to record the Disease prevalence and calculated using the formula [18]:

$$\text{Disease Prevalence} = \frac{\text{Total number of fields with stalk rot disease}}{\text{Total number of fields evaluated}} \text{X } 100$$

Disease Incidence

In all 10 maize growing districts surveyed, the disease incidence of stalk rot of maize was calculated during the year 2013, 2014 and 2015 by using formula [19]

Disease Incidence=(Number of Plants infected)/(Total number of plants evaluated)×100

Isolation of fungal pathogen associated with maize stalk rot

Maize stalks with stalk rot disease symptoms collected from maize fields of Karnataka were brought to the laboratory in paper bags and air-dried for 24 h. Samples were cut into small pieces (1cm) and surface sterilized with 2% Sodium Hypochlorite (NaOCl) solution for 2 - 3 min followed by three washes with sterile distilled water [20]. Samples were blotter dried and placed on potato dextrose agar (PDA; HiMedia, Mumbai-India) medium and incubated for 5-7 days at 28±2ºC. The growing fungal hyphal tips were transferred to PDA and grown for 2 days, and conidia were isolated using the single spore isolation method. Fungal colonies expressed after 7 days of incubation were identified based on micro-morphology of vegetative structures and fruiting bodies if any produced later.

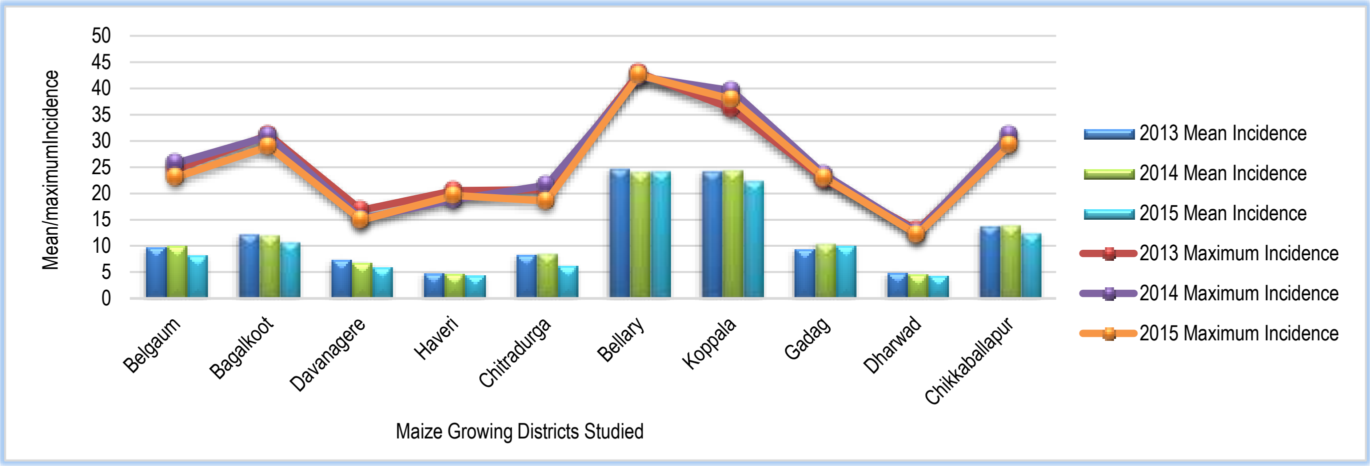

Fig-2 Mean Incidence and maximum incidence of Stalk rot disease of maize in the study regions (2013-15)

The fungal isolates were sub-cultured and stored at 4ºC for further studies. From each study site, a total of 20 representative stalk rot samples were collected and subjected for isolation of associated fungal pathogen. The isolation frequency of *Fusarium* species was determined by the number of samples having produced *Fusarium* species and the total number of samples plated using the formula provided by Mahadevakumar and Janardhana [21].

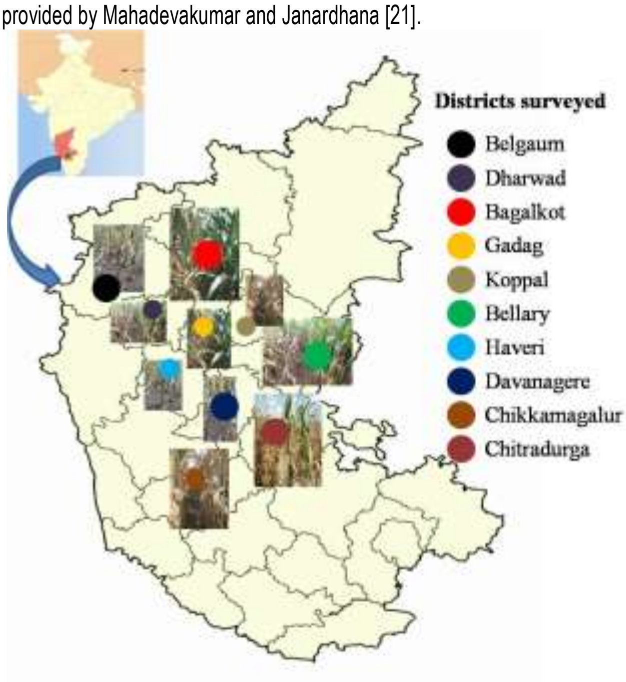

Fig-1 Map of Karnataka showing the study localities visited to assess stalk rot disease severity in Maize.

Table-3 Isolation frequency of *Fusarium* species from 10 major maize growing districts of Karnataka

SN	Districts	Number of Samples collected*			Isolation Frequency		
		2013	2014	2015	2013	2014	2015
1	Belgaum	20	20	20	70	48	40
2	Bagalkot	20	20	20	64	69	65
3	Davanagere	20	20	20	38	43	47
4	Haveri	20	20	20	50	45	55
5	Chitradurga	20	20	20	45	40	60
6	Bellary	20	20	20	42	52	48
7	Koppala	20	20	20	39	45	40
8	Gadag	20	20	20	67	42	49
9	Dharwad	20	20	20	65	30	60
10	Chikkaballapur	20	20	20	92	42	56
	Overall	200	200	200	57.2	45.6	52.0

Note: Stalk rot of Maize are used for isolation. *Value for total number of samples indicates the cumulative value for samples collected in survey period (2013 – 15).

Results

Disease prevalence and incidence of maize stalk rot

Maize stalk rot prevailed in all sites inspected and the degree of incidence varied from region to region and from year to year. The disease prevalence data collected along with total numbers of maize fields evaluated were presented in [Table-1]. Overall, a total of 705, 610 and 676 field were inspected during 2013, 2014 and 2015 respectively and the maximum prevalence (mean) was recorded during the *kharif* season of 2015 (56.4±17.02) followed by 2013 (53.71±19.23) and 2014 (50.87±17.88). Among the districts evaluated for the disease prevalence, highest mean prevalence of stalk rot was recorded during 2015 in Bellary (66.66%) followed by Chitradurga (66.50%) and Gadag (65.00%).

Fig-3 Isolation and cultural Characteristics of *Fusarium* species isolated from stalk rot samples of Maize from Karnataka: A – C: Plant tissue inoculated onto PDA expressed the development of *Fusarium sp.* colonies; D – I Pure cultures of *Fusarium sp.* isolated on PDA medium from the colonies expressed from maize stalk samples.

Incidence assessed in all the sites revealed that the disease was cause of severe reduction in productivity. The incidence varied from regions to regions and the data are presented in [Table-2]. Mean incidence was found maximum in Bellary and Koppala with >24% mean incidence followed by Ckikkaballapur and least mean incidence was recorded in Dharwad followed by Haveri [Table-2]]Fig-2]. Similarly, highest incidence of stalk rot was found in Bellary (Shankarbande-Bellary) followed by Koppala (Halagere-Koppala) in all three survey years (*kharif* season only) and minimum disease incidence was found in Dharwad followed by Davanagere.

Isolation frequency

The isolation frequency varied among the districts and season and the data are presented in [Table-3]. Highest frequency of *Fusarium* species was recorded from Belgaum (70%) followed Gadag (67%) and Dharwad (65%) in 2013. Similarly, highest *Fusarium* species frequency was recorded from Bagalkot (69%) followed by Bellary (52%) and Belgaum (48%) respectively; and in 2015, highest isolation frequency of 65% was observed in Bagalkot followed by Dharwad (60%) and Ckikkaballapur (56%) respectively.

Isolation and Identification of Fungal Pathogens

Among the samples screened for the isolation of associated fugal pathogen, a large number of samples produced *Fusarium* species and are the actual cause of maize stalk rot disease in all the sites. *Fusarium* species isolated from diseased stalk rot samples were characterized by the colonies which were fast growing, pale to bright-coloured with cottony aerial mycelium. The colour of the mycelium varied from whitish to purple [Fig-3]. The colonies typically produced both macro- and micro conidia from slender phialides. Macro-conidia are hyaline, two to several-celled, fusiform to sickle-shaped, with an elongated apical cell and pedicellate basal cell. Micro-conidia are one or two-celled, hyaline, smaller than macro-conidia, pyriform to ovoid, straight or curved [Fig-4]. Other important fungal pathogens associated with stalk rot disease are *Macrophomina phaseolina*; *Cephalosporium maydis*; *C. acremonium and Diplodia maydis*.

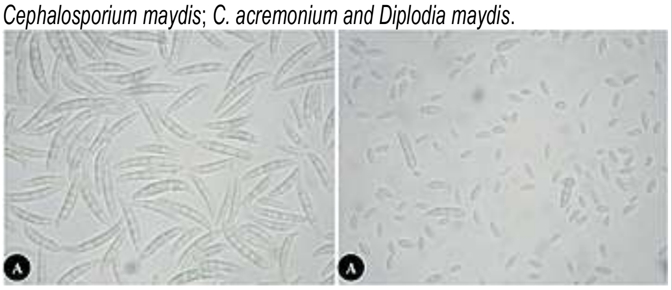

Fig-4 Microscopic images of *Fusarium* species isolated from stalk rot samples of Maize from Karnataka.

Discussion

Among major cereal crops in production, corn is the world's third leading crop after wheat and rice grown in different agro-ecologies of the world. It has highest genetic yield potential amongst the cereal crops. Diseases are one of the major constraints in realizing the potential yield of this crop. It suffers from several diseases but turcicum leaf blight (*Exserohilum turcicum*), maydis leaf blight (*Drechslera maydis*), polysora rust (*Puccinia polysora*), brown stripe downy mildew (*Sclerophthora rayssiae var. zeae*), sorghum downy mildew (*Peronosclerospora sorghii*), Rajasthan downy mildew (*Peronosclerospora heteropogoni*), banded leaf and sheath blight (*Rhizoctonia solani f spsasakii*), bacterial stalk rot (*Erwinia chrysanthemi* pv. zeae), post-flowering stalk rots (*Fusarium verticillioides, Macrophomina phaseolina*), Curvularia leaf spot (*Curvularia lunata*) are the important biotic constraints responsible for severe loss at global level [22-25]. Recently, a high incidence of stalk rot disease was observed in several maize fields of Karnataka State. The present study conducted in the Karnataka state covering 10 major maize growing districts to estimate the prevalence and incidence of stalk rot and it revealed that stalk rot is the major constraint in maize production. The severity varied from region to region and the associated fungal pathogen was dominated by species of *Fusarium.* Though there are other fungal pathogens associated with the stalk rot disease, *Fusarium* was important as it causes accumulation of toxins. Diseases in the pre-harvest stage are the major factors adversely affecting productivity in the study area. Post flowering stalk rots of maize is globally important destructive disease which affect the productivity by reducing the quality and quantity of productions. Post flowering stalk rot (PFSR) of maize is an important disease in India. It is a 'complex' disease as there is more than one pathogen was involved *viz. Fusarium verticillioides, Macrophomina phaseolina, Cephalosporium acremonium* and *Harpophora maydis* [26-28]. In the present study, several stalk rot samples were found to be associated with complex pathogens and combinations of fungal pathogens along with *Fusarium* specie are isolated. The most important ones are *M. phaseolina* and *C. acremonium*. In India, post flowering stalk rot was reported from Rajasthan, Uttar Pradesh, Bihar, Andhra Pradesh, West Bengal, Punjab and Madhya Pradesh, where there is scarcity of irrigation coupled with high soil temperature [29]. *Fusarium* species can infect any part of the maize plant from the beginning to the end of the growing season. Stalk rot is a common and severe disease on maize causing reduced growth, rotted leaf sheaths and internal stalk tissue death and brown streaks in the lower internodes. In mature plants, it causes pink to salmon discoloration of the internal stalk pith tissues [30]. The most important species of *Fusarium* associated with stalk rot disease of maize are *F moniliforme*, *F. temperatum* and *F. subglutinans*, belonging to the *Gibberella fujikuroi* species complex (GFSC), and *F. graminearum* (*G. zeae*) [21]. The symptoms of *Fusarium* stalk rot occur in warm and dry regions produces asexual spores and colonizes the internal tissues of stalk whereas the *Gibberella* stalk rot which occurs in cool, moist regions and produces tiny black perithecia which are sexual fruiting bodies of *Fusarium sp*. (*Gibberella* sp.) on infected maize stalks. Both the stalk rots results in premature death of maize plants by interfering with the translocation of water and nutrients to leaves and developing ears, causing yield losses [31-33]. The disease is characterized by disintegration of the pith tissue at or near the base of the stalk and is associated with senescence of stalk pith cells. Severe stalk rot results in yield loss through premature plant death and/or lodging. In addition, lodging of infected stalks makes harvesting difficult. In the tropics, about 40% of the fields are infested with *Fusarium* stalk rot with a severity level of about 10%. Stress conditions such as drought, excess water, lack of sunlight, leaf diseases, and insect damage, which reduce photosynthesis and the production of sugars, can predispose maize plants to severe stalk rot [34]. In conclusion, the study indicated that *Fusarium* species associated with maize stalk rot is distributed in all the major maize growing districts of Karnataka and considerable crop loss was recorded in all the regions. *Fusarium* species was the predominant fungal pathogen associated with stalk rot disease of maize. As the disease severity increased year after year in major maize growing regions, there is a dearth need for screening and identifying resistant sources against stalk rot disease. This will help in the long terms strategy to prevent crop losses due to *Fusarium* species infection. All the isolated *Fusarium* isolates were authenticated up to genus level based on the morphological features and cultural characteristics. However, identification of *Fusarium* species till species level is not possible as it requires utilization of molecular tools and techniques as many of the *Fusarium* species are cryptic in nature and morphology alone will not help in distinguishing the species. Therefore, further identification to species needs to be done by using molecular tools.

Application of research: The present research provides an insight into the stalk rot incidence and its impact on maize production in Karnataka. Further, identification and differentiation of associated fungal pathogens from wider geographical areas may give different species or organisms to be associated with stalk rot. As the stalk rot alone reduces the yield of up to 50% across the globe, it is more important to study the pathogen in detail.

Research Category: Plant disease, Plant Pathology

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Research Guide or Chairperson of research: Prof N. Lakshmidevi

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Research project name or number: PhD thesis

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment.

Study area / Sample Collection: Districts of Karnataka

Cultivar / Variety / Breed name: Maize (*Zea mays* L.)

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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