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ACKNOWLEDGEMENTS. We thank the Department of Science and Technology, Gov-

ernment of India for INSPIRE Fellowship (DST/INSPIRE Fellowship/2018/IF180050) to carry out this work. We also thank the Indian Coast Guard for ferrying us to Barren Island, and Chairman, ISRO for institutional support.

Received 13 January 2020; revised accepted 13 May 2020

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Occurrence of Nuclear Polyhedrosis Virus of invasive fall armyworm, *Spodoptera frugiperda* (J. E. Smith) in Meghalaya, North East India

The American fall armyworm (FAW), Spodoptera frugiperda (Smith) (Lepidoptera: Noctuidae) invaded Asia in 2018, causing huge damage to maize and other host crops in different countries¹. The invasive FAW is a highly polyphagous pest known to feed on 353 host plants across the globe². FAW was found in a severe form in maize fields of North East (NE) India after March 2019 (ref. 3). Due to distinct climatic conditions, NE India is considered the most diverse region of the country, which harbours a great diversity of flora and fauna. Moreover, NE India is organic by default and the majority of agricultural practices in the region are mainly natural and based on local resources. As a result, the region is being promoted by the Indian Government for natural/organic farming. Given the highly devastating nature of FAW, about 2-4 sprays of chemical pesticides are vital to reduce its population below the economic threshold level (ETL)⁴. However, due to limitations on the use of chemical pesticides for management of pests in the region, there is an urgent need to find cheaper, economically and ecologically viable options for the management of invasive pests like FAW.

Native strains or populations of natural biocontrol agents are reported to be more effective under such circumstances. Several entomopathogens, including viruses, fungi, protozoa, bacteria and nematodes are known to infect FAW life stages^{5–7}. In our experimental fields of maize at Umiam, Meghalaya, India, we found large-scale mortality of FAW larvae due to entomopathogenic fungus, *Metarhizium* (=*Nomuraea*) *rileyi* (Farlow) Samson and unknown pathogen symptomatically similar to Nuclear Polyhedrosis

Virus (NPV) of lepidopteran larvae. Later, the causal agent responsible for such symptoms in FAW appeared to be Spodoptera frugiperda Nuclear Polyhedrosis Virus (SpfrNPV). Due to humid climatic conditions, the M. rileyi is an important biocontrol agent of Spodoptera species in NE India. Therefore infection of M. rileyi to the larvae of related host species, i.e. S. frugiperda is expected. However, natural occurrence of SpfrNPV among FAW larvae in Meghalaya may provide additional opportunity to the farmers of the region to utilize this potential biocontrol agent against invasive FAW. Entomopathogenic viruses, especially NPVs are one of the most extensively studied and commercially exploited baculovirus group for pest management. FAW is basically infected by two baculovirus species, viz. NPVs (which are mainly multicapsid variants) and granulovirus⁸. Thus, SpfrNPV from Meghalaya is also expected to have multiple nucleocapsids in the virion of occlusion bodies (OBs), which can only be confirmed after detailed characterization. Worldwide, several NPVs have been isolated from FAW and several isolates have been used for the control of the pest with >80% efficacy, indicating its role as a potent biopesticide against this notorious pest⁹⁻¹².

Field observations of FAW in maize crop revealed that the SpfrNPV infected larvae stopped feeding and came out from the whorl on the leaf surface of maize plants. In certain cases, dead caterpillars were found hanging on the maize plants showing symptoms of typical NPV attack (Figure 1). Other diagnostic symptoms include sluggishness, initial lighter colour of the body which later becomes darkened, regurgitation and liquefaction of the body. Dead caterpillars of FAW (showing symptoms of typical NPV infection) were collected from the maize fields. Examination of the larval discharge in phase contrast illumination at 400× magnification showed typical polyhedral inclusion bodies of NPV (Figure 2). Using transmission electron microscopy, the aggregation of typical crystalline OBs was clearly observed in the discharge of SpfrNPV infected FAW caterpillars. Subsequently, a total of 687 caterpillars of FAW (second to fourth instars) were also collected from the maize fields of the locality and placed individually into the sterilized plastic containers (make: Tarsons[®]) for further development in the laboratory (at $25^{\circ} \pm 1^{\circ}$ C temperature, $75\% \pm 5\%$ relative humidity and 12:12light : dark period). Fresh primordial leaves of maize were provided as larval food throughout development. Maize leaves were surface-sterilized with an aqueous solution of sodium hypochlorite (0.05%) and cleaned with sterile distilled water to avoid external contamination through food. Among the field-collected larvae, 23.67% could not reach up to adult stage due to NPV infection. Surviving adults were allowed to mate and lay eggs on the substrate (filter paper) inside the oviposition cages. Honey solution was provided as adult food. After hatching from the eggs, 300 neonate larvae from the second generation were placed individually into sterilized plastic containers (make: Tarsons®) for further development on maize leaves. The FAW rearing process was continued until the third generation. The mortality trend was found to be increased in the second and third generations. A total of 39.33% and



Figure 1. *a*, Fall armyworm (FAW) larva infesting maize plants. *b*, Nuclear polyhedrosis virus infected FAW larva.



Figure 2. Polyhedral inclusion bodies (PIBs) of SpfrNPV visible under phase contrast illumination at 400× magnification.

48.67% mortality of early instars (second to fourth) were recorded during the second and third generations of FAW developed from the initial field-collected larvae. The overall pattern of viral disease incidence in FAW generations indicated more possibilities of either transovum (surface contamination of the eggs), or transovarial transmission (virus contained within the eggs) of viruses. Besides Meghalaya, SpfrNPV-infection has also been observed in FAW larvae in Gujarat, India¹³. Therefore, further detailed studies would give more insights on various characteristics of NPV infecting FAW in India. Considering the high specificity, virulence and environmental safety of the baculoviruses, several reports demonstrated that the different variants of NPV could be a good alternative to chemical pesticides for FAW management¹⁴; thus isolates are now

available commercially in some countries¹⁵. Further detailed studies on the efficacy of SpfrNPV under laboratory and field conditions are warranted to determine its potential against FAW.

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ACKNOWLEDGEMENTS. We thank the Director, ICAR Research Complex for North Eastern Hill Region, Umiam for providing the necessary facilities for this study, and Prof. V. K. Baranwal (ICAR-IARI, New Delhi) for help with electron microscopy. We also thank the three anonymous reviewers for their careful reading of the manuscript, and their insightful comments and suggestions.

Received 3 March 2020; revised accepted 12 May 2020

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