Norwegian University of Life Sciences
Faculty of Biosciences (Fakultet for Biovitenskap)
Department of Plant Sciences

Philosophiae Doctor (PhD)
Thesis 2022:73

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Ås, September 2022

Jeannette K. Winsou

Table of Contents

1 Abbreviations and definitions

AEZ	Agroecological zone
BC	Biological control
BIMAF	Biorisk Management Facility
BIOVIT	Fakultet for Biovitenskap (Faculty of Biosciences)
CSAT	Climate Smart Agricultural Technologies for improved rural livelihoods and Food Security in Mali and Niger
ECOWAS	Economic Community of West African States
FAW	Fall armyworm
FAO	Food and Agriculture Organization of the United Nations
IAS	Invasive Alien Species
icipe	International Centre of Insect Physiology and Ecology
IITA	International Institute of Tropical Agriculture
ILCYM	Insect Life Cycle Modelling
IPM	Integrated Pest Management
NCSU	North Carolina State University
NIBIO	Norsk institutt for bioøkonomi (Norwegian Institute for Bioeconomy Research)
NMBU	Norges miljø- og biovitenskapelige universitet (Norwegian University of Life Sciences)
NORAD	Norwegian Agency for Development Cooperation
R4D	Research for Development
RCN	Research Council of Norway
SLU	Swedish University of Agricultural Sciences
WCA	West and Central Africa

2 List of papers

The PhD thesis contains the following papers:

I. Jeannette K. Winsou, Ghislain T. Tepa-Yotto, Karl H. Thunes, Richard Meadow, Manuele Tamò and May-Guri Sæthre 2022. **Seasonal variations of *Spodoptera frugiperda* host plant diversity and parasitoid complex in southern and central Benin**

II. Jeannette K. Winsou, Ghislain T. Tepa-Yotto, Karl H. Thunes, Richard Meadow, Manuele Tamò and May-Guri Sæthre 2022. **Life history parameters of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) on two different host plants at ambient temperatures: implications on population dynamics during maize off-seasons in southern Benin (West Africa)**

III. Jeannette K. Winsou, Ghislain T. Tepa-Yotto, Karl H. Thunes, Richard Meadow, Manuele Tamò and May-Guri Sæthre 2022. **Phenology and functional response of the egg parasitoid *Telenomus remus*: comparison of local and exotic parasitoid strains at three constant temperatures for biocontrol decision support**

IV. Jeannette K. Winsou, Ghislain T. Tepa-Yotto, Karl H. Thunes, Richard Meadow, Manuele Tamò and May-Guri Sæthre 2022. **Assessment of three release methods of *Telenomus remus* to control *Spodoptera frugiperda* in southern Benin: field cage experiments**

3 Summary

Since its outbreak in 2016 *Spodoptera frugiperda* (Lepidoptera: Noctuidae) commonly known as fall armyworm (FAW) has caused significant consequences on a range of crops, especially on maize, which is a staple food in most sub-Saharan African countries. FAW is currently reported to be the most destructive Invasive Alien Species (IAS) on the continent with the highest yield losses amounting to USD 9.4-13 billion. In Benin, estimations from a farmer survey of yield losses due to the pest reached 49% in maize systems. The present thesis discusses biological control using egg parasitoids as one of the safest IPM solutions against insect pests, with a goal of reduced use of chemical control. To achieve this goal in the context of FAW outbreaks in Benin, the thesis included a systematic assessment of drivers for successful biological control. The specific objectives were to i) document the host plant range of *S. frugiperda* and the parasitoids adapting to the pest in southern and central Benin; ii) compute the life history parameters of *S. frugiperda* when fed with maize and one of the most frequently recorded alternative host plant (onion) during the larval stage at ambient fluctuating temperatures; iii) compare the performance of two strains of the egg parasitoid *Telenomus remus* Nixon (Hymenoptera: Scelionidae) on *S. frugiperda*; iv) assess three parasitoid release methods on the performance of the locally recruited egg parasitoid *T. remus* under field cages. Field surveys were conducted in southern and central Benin to sample host plants associated with FAW and parasitoids adapting to the pest. Laboratory investigations were carried out to examine both pest and parasitoid (*T. remus*) life tables. Three parasitoid release methods of the local strain of *T. remus* were examined in field cages to determine the most efficient against FAW.

Out of the 29 alternative host plant species of FAW recorded, eleven new host plant species were reported in this study compared to earlier surveys. Aside from maize, the prime host plant of FAW, onion (*Allium cepa*) was frequently found associated with the pest in the field. The net reproductive rate (R_o), the intrinsic (r_m) and finite (λ) rates of increase measured in the laboratory were relatively higher on maize (499.92; 0.27 and 1.32) compared to onion (63.77; 0.13 and 1.14), respectively. The development of FAW fed on onion at the larval stage was delayed at ambient temperatures.

Seven parasitoid species belonging to four families were found on *S. frugiperda*. The egg parasitoid *Telenomus remus* was the most abundant species in the field. The performance of two strains (local and exotic) of *T. remus* was measured in the laboratory. The local female parasitoid strain was relatively the most prolific at 31 °C, with on average 159.0 FAW eggs parasitized per female parasitoid. Female lifetime fecundity was equal between the two parasitoid strains at 25 °C ($F_{1,28} = 1.18$; $P = 0.286$), 28 °C ($F_{1,25} = 0.02$; $P = 0.884$) and 31 °C ($F_{1,25} = 0.15$; $P = 0.694$). Temperature did not affect the exotic parasitoid strain's lifetime fecundity ($F_{2,40} = 0.05$; $P = 0.949$), nor for the local parasitoid strain, with the highest number of FAW eggs parasitized per female parasitoid at 31 °C ($F_{2,38} = 1.07$; $P = 0.355$). The number of eggs parasitized increased while the percentage of eggs parasitized was inversely proportional to the FAW egg density, displaying type II functional response patterns.

Increased female density beyond 5 wasps did not significantly improve *T. remus* performance on clusters of 100-200 FAW eggs. Three parasitoid release methods were compared under field cages in a randomized complete block design with four treatments and four replicates; method 1) weekly parasitoid release until the maize tasseling stage; method 2) three consecutive parasitoid releases at three-day intervals; and method 3) parasitoid release at V4 and V10 stages of maize growth. The three parasitoid release methods of the local strain tested were statistically comparable. None of the experiments revealed significant differences between the methods in both major and minor cropping seasons. However, method 1 (weekly releases) was the most efficient in the minor season, which was not the case during the major season where method 3 (release at V4 and V10) was the best. The current work provides pioneering information on the potential of *Telenomus remus* which could be deployed in augmentation and conservation biological control programs in Benin.

4 Norsk sammendrag

Siden det første utbruddet i 2016 har *Spodoptera frugiperda* (Lepidoptera: Noctuidae), også kjent som Fall Armyworm (FAW) forårsaket alvorlige ulemper for en rekke matvekster, spesielt for mais som er hovednæringsvekst i de fleste land sør for Sahara. FAW er i disse dager meldt å være den mest ødeleggende fremmed- og invaderende arten (FIA) på kontinentet, med avlingstap estimert til 9.4-13 milliarder USD. I Benin rapporterte en undersøkelse av avlingstap blant bønder forårsaket av skadegjøreren til 49% i maissystemer. Denne avhandlingen omhandler biologisk kontroll ved å benytte parasitoider på egg som en av de tryggeste IPV behandlinger mot insektskadegjørere og har som mål å redusere bruken av kjemisk kontroll. For å nå dette målet i sammenheng med utbruddene av FAW i Benin, ble det i denne avhandlingen foretatt en systematisk gjennomgang av driverne for vellykket biologisk kontroll. De spesifikke målene var i) å dokumentere bredden av vertsplanter for *S. frugiperda* og parasitoidene som tilpasser seg skadegjøreren i det sørlige og sentrale Benin; ii) beregne de viktigste parameterne for livshistorien til *S. frugiperda* der larver ble foret med henholdsvis mais og en av de vanligste alternative vertsplanter (løk), og ved omgivelsestemperatur; iii) sammenligne hvordan to varianter av eggparasitoiden *Telenomus remus* Nixon (Hymenoptera: Scelionidae) opptrer på *S. frugiperda*; iv) vurdere tre metoder for kontrollert utsetting av den lokale varianten av eggparasitoiden *T. remus* i feltkasser. Feltforsøk for å samle inn vertsplanter assosiert med FAW og parasitoider som tilpasser seg til skadegjøreren ble gjennomført i det sørlige og sentrale Benin. Laboratorieforsøk ble utført for å undersøke livshistoriene til både skadegjøreren og parasitoiden (*T. remus*). Tre utsettingsmetoder av den lokalt forekommende varianten av *T. remus* ble gjennomført i feltkasser for å undersøke hvilken av dem som var mest effektiv mot FAW.

Av de 29 alternative vertsplantene for FAW som ble registrert, elleve nye vertsplanter ble rapportert i dette arbeidet sammenlignet med tidligere undersøkelser. I tillegg til hovedverten for FAW ble løk, *Allium cepa,* ofte funnet assosiert med skadegjøreren ute i felt. Netto reproduksjonsrate (R_0), egen (r_m) og endelig (λ) økningsrate målt i laboratoriet var relativt sett høyere hos mais (499.92; 0.27 and 1.32) sammenlignet

med løk (63.77; 0.13 and 1.14). Utviklingen hos FAW som ble foret med løk var forsinket ved omgivelsestemperatur.

Syv arter parasitoider tilhørende fire familier ble funnet på *S. frugiperda*. Eggparasitoiden *Telenomus remus* var den vanligste i felt. Hvordan to varianter (lokal og eksotisk) av *T. remus* fungerte ble målt i laboratoriet. Den lokale parasitoidvarianten var relativt sett mest produktiv ved 31 °C og med gjennomsnittlig 159.0 parasitterte FAW egg per parasitoid hunn. Livsløpfekunditet per hunn var lik mellom de to parasitoidvariantene ved 25 °C ($F_{1,28} = 1.18$; $P = 0.286$), 28 °C ($F_{1,25} = 0.02$; $P = 0.884$) og 31 °C ($F_{1,25} = 0.15$; $P = 0.694$). Temperatur hadde ingen effekt på den eksotiske variantens livsløpfekunditet ($F_{2,40} = 0.05$; $P = 0.949$), heller ikke for den lokale varianten med det høyeste antallet parasitterte FAW egg per hunn ved 31 °C ($F_{2,38} = 1.07$; $P = 0.355$). Antall parasitterte egg økte samtidig med at andel parasitterte egg var omvendt proporsjonalt med tettheten av FAW egg, noe som viser en type II funksjonell respons.

Økt tetthet av hunner utover 5 vepser ga ingen signifikant forbedring av effektiviteten av *T. remus* på eggmasser med 100-200 FAW egg. Tre utsettingsmetoder ble sammenlignet i feltkasser i en randomisert komplett blokkdesign med fire behandlinger og fire replikasjoner; metode 1) ukentlig utsetting av parasitoider inntil dusken på maiskolben kom frem; metode 2) tre påfølgende utsettinger av parasitoider hver tredje dag; og metode 3) utsetting av parasitoider ved vekststadium V4 og V10 hos mais. De tre utsettingsmetodene var statistisk sammenlignbare. Ingen av eksperimentene viste signifikante forskjeller mellom metodene hverken i hoved- eller sekundæresesongen for dyrking. Likevel, metode 1 (ukentlig utsetting) var mest effektiv i sekundærsesongen, i motsetning til hovedsesongen hvor metode 3 (utsetting ved stadium V4 og V10) var den beste. Dette arbeidet leverer grunnleggende informasjon om potensialet *Telenomus remus* har for augmentativ og konserverende biologiske kontrollprogram i Benin.

5 Synopsis

5.1 Introduction

Agriculture is the most important economic sector in Africa. It also constitutes the main sector of employment with one out of two working people in rural areas of sub-Saharan African countries. Increased productivity in this sector of activity will secure food security and is a source of direct household income, especially in West Africa (FAO 2020, Eschen et al. 2021). However, many challenges, such as pests of cereals could jeopardize the agricultural potential. In 2016, outbreaks of *Spodoptera frugiperda* (Lepidoptera; Noctuidae) commonly known as fall armyworm (FAW), were recorded for the first time in west and central Africa. It had significant impact on a range of crops, especially on maize which is a staple food in most of sub-Saharan countries on the continent (Goergen et al. 2016, FAO 2022a). To date FAW has been reported in 78 countries in Africa, the Near East, Asia and the Pacific. While it was initially found on a few plant species in a few African countries, dozens of additional species, either cultivated crops or wild host plants were further discovered hosting FAW (FAO 2022b, Winsou et al. 2022). FAW is currently reported to be the most destructive Invasive Alien Species (IAS) in sub-Saharan Africa with the highest yield losses amounting to USD 9.4-13 billion (Abrahams et al. 2017, Eschen et al. 2021). In Benin, estimations from a farmer survey of yield losses due to the pest reached 49% in maize systems (Houngbo et al. 2020). This triggered the deployment of a range of interventions against FAW including successful integrated pest management (IPM) tactics tested in eight geo-zones, the use of hybrid maize tolerant to FAW and many synthetic insecticides and biopesticides registered for FAW control in Africa (FAO 2022b, Tepa-Yotto et al. 2022). However, FAW invasion still continues putting tens of millions of people at risk and may worsen food insecurity and lead to fragile livelihoods in Africa in the absence of sustainable measures that can be successfully applied by farmers (FAO 2022b).

Therefore, comprehensive assessments of the pest status along with practical and adaptable IPM options are required.

The integrated pest management (IPM) strategy is a rational combination of plant protection options which includes, but is not limited to chemical control, host plant resistance, biopesticide use, agroecologically based management practices and biological control (Eilenberg et al. 2001). However, the farmer's first line solution that is available and applied to face the pest attack is often the use of synthetic insecticides. Unfortunately, the sole use of insecticide as a standalone pest control strategy is unsustainable and associated with potential adverse human health and environmental hazards and insect resistance. Thus, there is a need to promote durable techniques including biological control.

Biological control (BC) as an important IPM component is the use of living organisms to suppress the population of a specific pest organism, making it less abundant or less destructive than it would otherwise be. Biological control encompasses four approaches: (1) classical biological control, (2) inoculation biological control, (3) inundation biological control, and (4) conservation biological control (Eilenberg et al. 2001).

The present thesis discusses biological control as one of the safest IPM solutions against insect pests with a view of reduced use of chemical control. To achieve this goal in the context of FAW outbreaks in Benin, we performed a systematic assessment of drivers for successful biological control using an adjusted approach based on a set of indicators of the feasibility of biocontrol from Barbosa and Segarra-Carmona (1993) and Tamo et al. (1997). P**aper I** focuses on the seasonal variations of the *Spodoptera frugiperda* host plant diversity and parasitoid complex in southern and central Benin. We undertook surveys of FAW host plants and local parasitoids which have adapted to the pest during maize growth season and off-season. In-depth examination of the ability of FAW to maintain its offspring on alternative host plants was carried out for **paper II**, by calculating the life history parameters of the pest when fed with maize as the prime host plant and onion leaves as an alternative host plant during the larval stage at ambient fluctuating temperatures. **Paper III** analyses the biological characteristics and life table parameters of a promising biocontrol agent, the egg parasitoid *Telenomus remus* Nixon (Hymenoptera: Scelionidae) (Kenis et al. 2019) which has adapted to FAW in Benin. We compared the life table parameters of local and exotic strains of the egg parasitoid *T. remus.* In **paper IV**, parasitism performance investigations of the locally recruited egg parasitoid *T. remus* against FAW were done in field cages. This topic also compared various parasitoid release methods of the egg parasitoid to support decision making for biocontrol programs.

5.2 Background

5.2.1 Global distribution and recent spread of fall armyworm

Fall armyworm (FAW), *Spodoptera frugiperda* is a lepidopteran noctuid native to the tropical and subtropical regions of the Americas where it has been an important crop pest for centuries. FAW was first recognized by Smith and Abbot in 1797 who gave the first of the numerous common names ''corn-bud-worm-moth''. The pest underwent a few taxonomic reviews and name changes. Initially named *Phalaena frugiperda* in 1797 by Smith and Abbot, the pest was further identified as *Prodenia autumnalis* var *fulvosa* by Riley 1970. The current scientific name *Spodoptera frugiperda* depicts the high voracious and destructive nature of the pest (the whole fruit is lost). The multiple and periodic outbreaks of this pest ranked it an important economic threat in the Americas (Luginbill 1928, Gebreziher 2020). *Spodoptera frugiperda* does not undergo diapause but has a great migratory capability (Nagoshi et al. 2012). The adults of *S. frugiperda* can migrate hundreds kilometers overnight (Day et al. 2017, FAO 2022b) and its populations are capable of traveling northwards thousands kilometers yearly from South America and die during harsh winters (Vatanparast and Park. 2022).

FAW was first detected in early 2016 in west and central Africa, namely in Benin, Nigeria, Sao Tome and Principe, and Togo (Goergen et al. 2016). It was later reported in the whole of southern Africa's inland except Lesotho, in Madagascar and the Seychelles. The pest quickly spread across almost all of sub-Saharan Africa (SSA) two years post-detection (Sisay et al. 2018, Njuguna et al. 2021, FAO 2022a). As of July 2018, it was reported in India and Yemen (Sharanabasappa et al. 2018). In December 2018, the pest occurred in Bangladesh, Sri Lanka and Thailand. By November 2019, FAW was also found in Sudan, Egypt, Yemen, and in many Asian countries including India, Bangladesh, Sri Lanka, Thailand, Myanmar, China, Indonesia, the Philippines, Laos, Malaysia, Viet Nam, Cambodia, the Republic of Korea and Japan. Between February and May 2020, it was detected in Australia, Mauritania, Timor Leste and the United Arab Emirates. In late 2020, it was confirmed in Jordan, Syria and Papua New Guinea. In January 2021, New Caledonia reported FAW and by April it entered the Canary Islands of Spain (FAO 2022a). To date FAW is recorded in 78 countries including almost the entire continent of Africa, the Near East, Asia and the Pacific (FAO 2022b) (Figure 1).

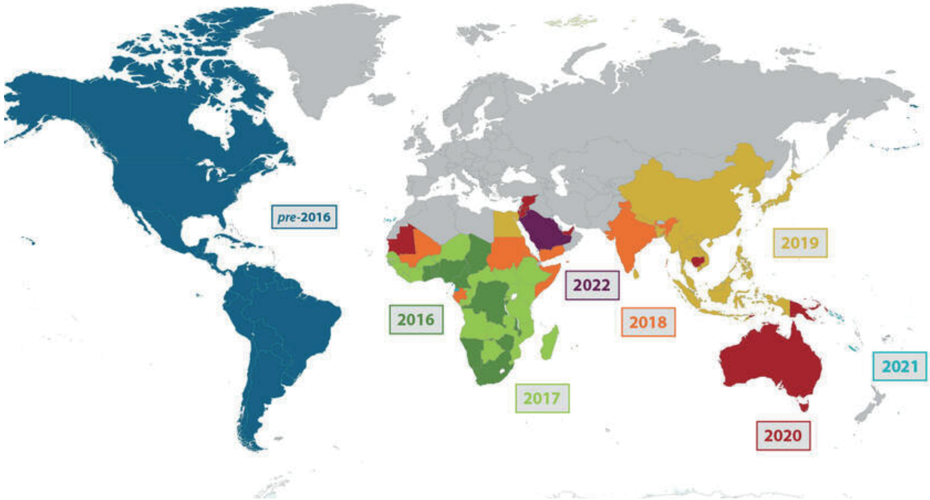


Figure 1. Global distribution of FAW as of 2022 (FAO 2022a).

5.2.2 Fall armyworm biology

The adults of *S. frugiperda* have a wingspan of 32 to 40mm. The male forewing is generally shaded grey and brown with white spots at the tip near the center while the female forewing pattern range from uniform grayish brown to a fine mottling of gray and brown. The female forewing is generally shaded gray and brown. Both female and male hind wing is iridescent silver-white with a narrow border (Huesing et al. 2018). Fall armyworm adult male (Figure 2) outer wings have whitish patches at the lower outer edges while the inner wings are white with dark trimmings (CABI, 2019). The female's outer wings are uniform shining grayish (CABI, 2019). FAW develops through four major stages: egg, larva, pupa and adult (Figures 3 & 4). The female lays eggs in masses which contain about 100 to 200 individuals each and in its lifetime it lays 1500 to 2000 eggs. The egg is dome shaped and measures about 0.4mm in diameter and 0.3mm in height. Its colour varies between blackish, white and pinkish. The egg stage duration is 2 to 3 days in warm temperatures. FAW has six larval instars which vary in color and head capsule dimensions. The larval stage is typically characterised by ''four-dot square'' in the posterior part of the larval dorsal face and a light-coloured inverted ''Y' on the face of the mature larva (Huesing et al. 2018, CABI, 2019). The pest is destructive at its larval stage. As a noctuid *Spodoptera frugiperda*'s young larvae hide in the maize whorl during the day and emerge at night to feed on the plant (CABI, 2019). Older larvae dwell inside the maize whorl and are responsible for critical damage particularly on reproductive plant organs (Day et al. 2017). Fall armyworm is cannibalistic at its larval stage and most likely at the third instar stage, which explains frequent cases of solitary larval behaviour on the maize plants in the field. The pupae measure 14 to 18cm and their colour varies from reddish to brownish. The pupal stage is approximately 8-14 days long in warm conditions (Prasanna et al. 2018). FAW pupate normally in the soil by constructing a loose cocoon at a depth of 2 to 8cm or using other materials such as leaves if the soil is hard. The pest does not have a diapause mechanism and is therefore unable to survive when the environmental conditions are not favourable (extreme cold and heat). The average duration of the adult stage is 10 days and can vary between 7 -21 days (Huesing et al. 2018, CABI, 2019).

FAW is a polyphagous pest which feeds on both vegetative and reproductive structures of the host. The most severe attack of FAW is often observed on the growing point which results in "dead heart", wilting and death of the unfurled leaves (Day et al. 2017). On maize, the damage can be observed on all parts of the plant and the constant feeding can result in skeletonized leaves (Goergen et al. 2016). However, some studies reported a partitioning in time and space of the maize plant development stage between the indigenous stemborer species and FAW showing the coexistence of both pests with FAW on the vegetative phase while the stemborers attack the plant at the reproductive stage. The co-occurrence of both aggravates damage on maize in absence of control measures (Abang et al. 2021). This can also result in a displacement of the native species, similar to the well-known example from Africa of the native stemborer *Chilo orichalcociliellus* Strand (Lepidoptera: Crambidae) by the invasive stemborer *C. partellus* Swinhoe (Lepidoptera: Crambidae) which became in the end more destructive in south and east of the continent (Mutamiswa et al. 2017). Varying yield loss estimates due to FAW damage have been reported by several authors in a few sub-Saharan African countries. These estimates can be categorized as farmer perception-based approximations (Houngbo et al. 2020), socioeconomic survey findings (De Groote et al. 2020) and direct empirical yield-loss records in maize fields (Baudron et al. 2019). Overall, FAW damage can negatively affect the availability of seed for the next maize-season (Prasanna et al. 2018) pointing to the need for effective management strategies.

5.2.3 Fall armyworm host plants

FAW is recorded in its origin on a number of crop species including wheat, sorghum, millet, sugarcane, vegetables crops and cotton, with maize as its prime host plant (FAO 2022b). The pest has a wide host plant range either cultivated or wild with a great affinity to the graminaceous (Figures 5 & 6) (Montezano et al. 2018, FAO 2022b, Winsou et al. 2022). According to Montezano et al. (2018), FAW's host plants range is up to 353 plants belonging to 76 plant families. The alternative host plants ensure pest propagation the year round (Nyamutukwa et al. 2022, Winsou et al. 2022).

Figure 2. *Spodoptera frugiperda* adult moth opened wings (A) and resting position (B). Photo credit: (A) Georg Goergen, IITA; (B) Matt Bertone, NCSU.

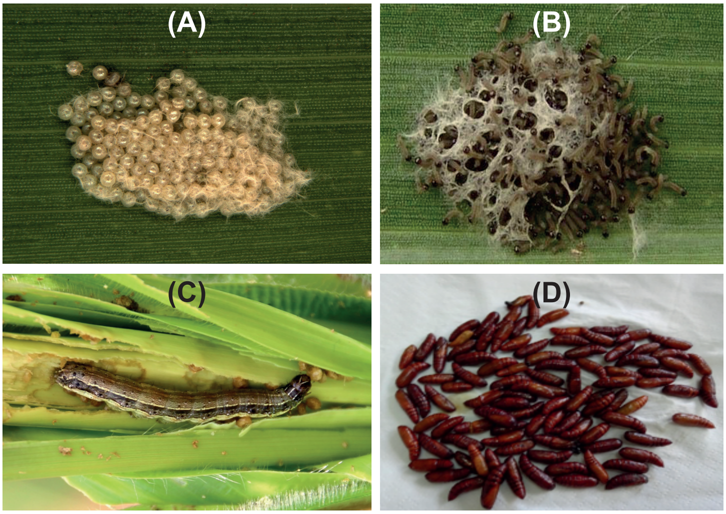
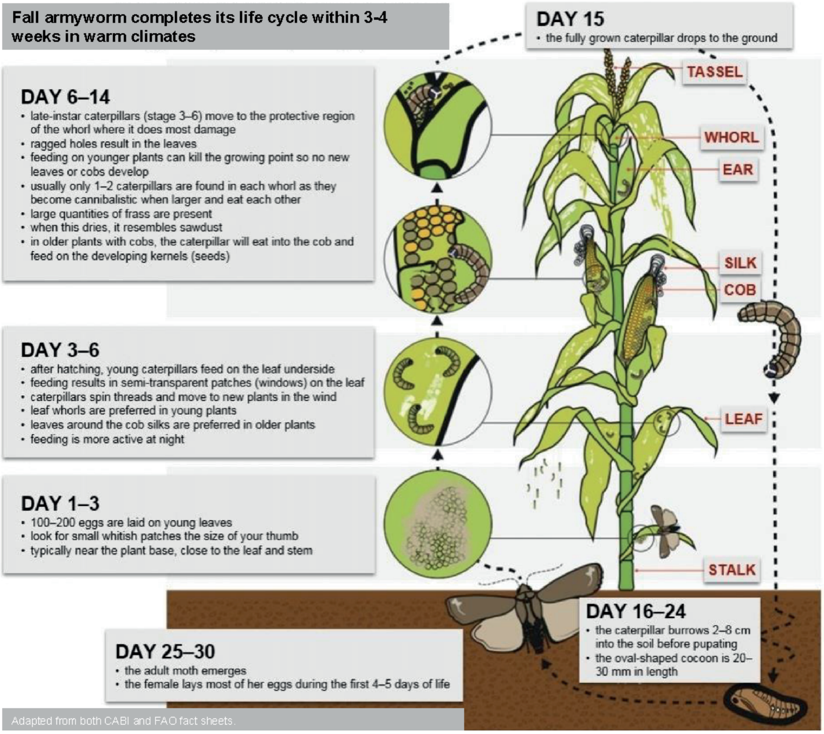


Figure 3. Fall armyworm immature stages, (A) egg mass, (B) neonates, (C) sixth instar larva and (D) pupae. Photo credit: (A) Georg Goergen, IITA; (B) Ivan Cruz, EMBRAPA; (C) & (D) Jeannette K. Winsou, NMBU.



Fall armyworm completes its life cycle within 3-4 weeks in warm climates

DAY 15
- the fully grown caterpillar drops to the ground

DAY 6–14
- late-instar caterpillars (stage 3–6) move to the protective region of the whorl where it does most damage
- ragged holes result in the leaves
- feeding on younger plants can kill the growing point so no new leaves or cobs develop
- usually only 1–2 caterpillars are found in each whorl as they become cannibalistic when larger and eat each other
- large quantities of frass are present
- when this dries, it resembles sawdust
- in older plants with cobs, the caterpillar will eat into the cob and feed on the developing kernels (seeds)

DAY 3–6
- after hatching, young caterpillars feed on the leaf underside
- feeding results in semi-transparent patches (windows) on the leaf
- caterpillars spin threads and move to new plants in the wind
- leaf whorls are preferred in young plants
- leaves around the cob silks are preferred in older plants
- feeding is more active at night

DAY 1–3
- 100–200 eggs are laid on young leaves
- look for small whitish patches the size of your thumb
- typically near the plant base, close to the leaf and stem

DAY 16–24
- the caterpillar burrows 2–8 cm into the soil before pupating
- the oval-shaped cocoon is 20–30 mm in length

DAY 25–30
- the adult moth emerges
- the female lays most of her eggs during the first 4–5 days of life

Adapted from both CABI and FAO fact sheets.

Figure 4. FAW life cycle (adapted from both CABI and FAO fact sheets) (CABI, 2019)

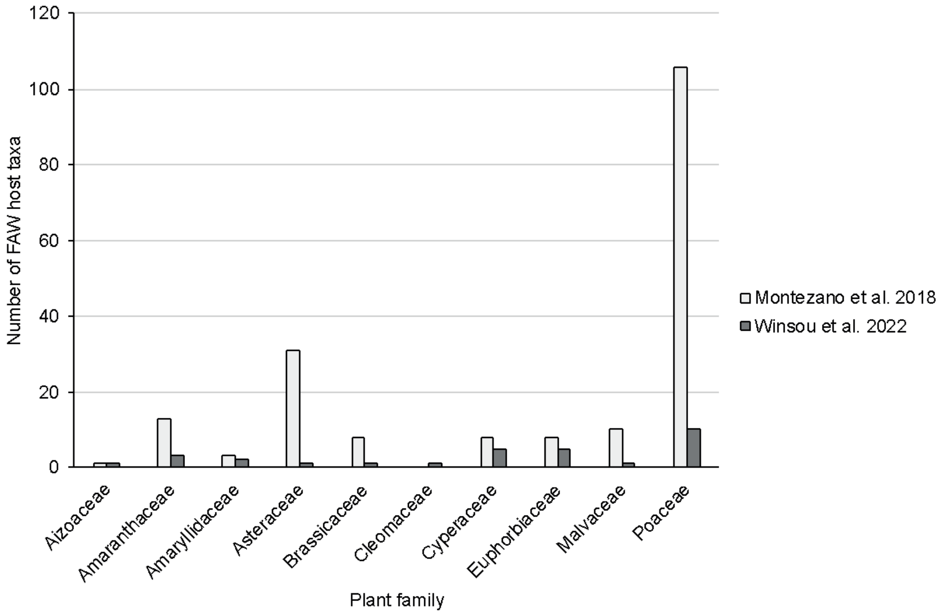


Figure 5. Fall armyworm host plant species records in the Americas (Montezano et al. 2018) and in Benin (Winsou et al. 2022). Only records from the ten plant families found by Winsou et al. 2022 six years post pest detection in Benin are aggregated. More plant families were reported by Montezano et al. 2018.

Figure 6. FAW larva on alternative host plants (A) *Allium cepa* L. and (B) *Saccharum officinarum* L. Photo credit: Jeannette K. Winsou, NMBU.

5.2.4 Fall armyworm parasitoids

The pest natural enemies can be globally divided in three major categories, which are predators, parasitoids, and entomopathogenic microorganisms. The current work focuses on the various parasitoids which have adapted to the pest since its outbreaks in Africa in 2016 (Table 1). FAW parasitoids encompass egg, larval, egg-larval, pupal and larval-pupal parasitoids. Reports in a few African countries confirm some complexes of locally recruited parasitoids which have adapted to FAW. To date and to the best of our knowledge only a few parasitoid species were associated with FAW materials collected on alternative/wild host plants in Africa (Winsou et al. 2022). Moreover, there are some surveys showing the existence of shared local parasitoids between FAW and resident stemborer communities. These parasitoids include *Telenomus remus* (egg parasitoid), *Cotesia icipe* (larval parasitoid) and *Procerochasmias nigromaculatus* (pupal parasitoid) (Abang et al. 2021).

Table 1. Locally recruited parasitoids which have adapted to FAW since the pest outbreaks in West and Central Africa in 2016.

Order, family and species	**Types**	**Countries**	**FAW host plant Associated**	**References**
Hymenoptera: Scelionidae				
Telenomus remus Nixon	Egg parasitoid	Benin, Cameroon, Côte d'Ivoire, Ghana, Kenya, Niger, South Africa, Tanzania	Maize, *Panicum maximum, Sorghum* sp.	(Kenis et al. 2019, Sisay et al. 2019, Abang et al. 2020, Agboyi et al. 2020, Laminou et al. 2020, Winsou et al. 2022)
Trichogrammatidae				
Trichogramma chilonis Ishii	Egg parasitoid	Cameroon, Kenya	Maize	(Sisay et al. 2019, Abang et al. 2020)
Trichogramma sp.	Egg parasitoid	Benin	Maize	(Agboyi et al. 2020)
Trichogrammatoidea	Egg parasitoid	Niger	Sorghum	(Laminou et al. 2020)
Diptera: Chloropidae				
Anatrichus erinaceus Loew	Larval parasitoid	Ghana	Maize	(Koffi et al. 2020)
Tachinidae				
Drino quadrizonula Thomson	Larval parasitoid	Ghana, Benin	Maize	(Agboyi et al. 2020, Winsou et al. 2022)
Palexorista zonata (Curran)	Larval parasitoid	Ethiopia, Kenya	Maize	(Sisay et al. 2018, 2019)

Table 1. *Cont.*

Order, family and species	Types	Countries	FAW host plant Associated	References
Undetermined sp.	Larval parasitoid		Maize	(Koffi et al. 2020)
Hymenoptera: Braconidae *Chelonus* sp.	Larval parasitoid	Senegal	Maize	(Tendeng et al. 2019)
Chelonus bifoveolatus Szépligeti	Egg-larval parasitoid	Benin, Ghana	Maize, *Andropogon* sp., *Bulbostylis coleotrica, Panicum maximum*	(Agboyi et al. 2020, Winsou et al. 2022)
Chelonus curvimaculatus Cameron	Egg-larval parasitoid	Kenya	Maize	(Sisay et al. 2018, 2019)
Mateoridea testacea (Granger)	Egg-larval parasitoid	Benin, Ghana	Maize	(Agboyi et al. 2020, Koffi et al. 2020)
Coccygidium luteum (Brullé)	Larval parasitoid	Benin, Ghana, Ethiopia, Kenya, Tanzania	Maize; *Digitaria* cf. *horizontalis; Allium cepa; Sorghum arundinaceum; Cyperus* sp.; *Panicum maximum*	(Sisay et al. 2018, 2019, Agboyi et al. 2020, Winsou et al. 2022),
Cotesia cesamiae (Cameron)	Larval parasitoid	Cameroon	Amaranthus, maize	(Abang et al. 2021)
Cotesia icipe Fernandez-Triana and Fiobe	Larval parasitoid	Benin, Ethiopia, Ghana, Kenya, Tanzania	Maize*; Amaranthus spinosus; Bulbostylis coleotrica; Allium cepa; Sorghum arundinaceum*	(Sisay et al. 2018, 2019, Abang et al. 2020, Koffi et al. 2020, Winsou et al. 2022)
Hymenoptera: Ichneumonidae *Charops* sp.	Larval parasitoid	Benin, Ghana	Maize; *Digitaria* sp.; *Allium cepa; Bulbostylis coleotrica; Andropogon* sp.	(Agboyi et al. 2020, Winsou et al. 2022)
Charops ater Szépligeti	Larval parasitoid	Kenya,Tanzania	Maize	(Sisay et al. 2018)
Metopius cf discolor (Tosquinet)	Larval parasitoid	Ghana	Maize	(Agboyi et al. 2020)
Campoletis sp.	Larval parasitoid	Senegal	Maize	(Tendeng et al. 2019)
Pristomerus pallidus (Kriechbaumer)	Larval parasitoid	Benin	Maize*; Cyperus roduntus; Sorghum arundinaceum*	(Agboyi et al. 2020, Winsou et al. 2022)
Hymenoptera: Ichneumonidae *Procerochasmias nigromaculatus* (Cameron)	Pupal parasitoid	Cameroon	Maize	(Abang et al. 2020)

5.3 Status of knowledge

5.3.1 Maize importance and knowledge gaps

Maize is the cereal most widely grown across diverse agroecological zones (AEZs) in Africa and used as staple food by around half the population of the African continent (Day et al. 2017). Maize as a staple food is consumed in various dishes from raw to improved culinary transformed food, which contributes to food and nutritional security for over 200 million people (Day et al. 2017).

Maize is the most produced cereal in West Africa, but its production is hindered by many pests including stemborers, termites, silkworms, grasshoppers and weevils (Nafiu et al. 2014, Abang et al. 2021). Stemborers including African stemborer *Busseola fusca,* the pink stemborer *Sesamia calamistis* Hampson, the African sugarcane stemborer *Eldana saccharina* Walker *and Chilo partellus* Swinhoe are associated with maize yield losses in Africa, but only the sudden outbreaks of *Spodoptera exempta* (Walker) involved significant control measures (Chabi-Olaye et al. 2005, Hailu et al. 2021, Chinwada et al. 2022, Tepa-Yotto et al. 2022, Chinwada, et al. 2022). Among the native pests, only *Busseola fusca* Fuller (Lepidoptera: Noctuidae) has for a long time been the most destructive pest of maize in Africa (Abang et al. 2021). Since 2016 FAW outbreaks with resulting alarming damage on maize, additional burden to the already existing agricultural challenges stimulated a continental engagement at every level to tackle the pest problem (Goergen et al. 2016).

The protection of maize against pests involves a range of control measures including chemical control, host plant resistance, agroecological management practices, biological control and indigenous practices. Many indigenous practices were used by the smallholder farmers during the outbreak of FAW. These practices included physical and mechanical strategies such as crushing the egg masses and neonates, putting sand or wood ash inside plant funnels, and the use of laundry-washing powders to drench the plant funnels. The application of some extracts of plants such as neem (*Azadirachta indica*) and velvet bean (*Mucuna pruriens*), and fish soup was also used against FAW.

Some cultural practices such as push-pull techniques and crop intercropping were also part of the numerous options used for FAW management (Hailu et al. 2018, Murray et al. 2019, Aniwanou et al. 2020, Niassy et al. 2021). The management of FAW also considered the use of multiple chemicals which include the synthetic insecticides to which FAW has already developed resistance in the Americas such as 1B, 3A organophosphates and most of pyrethroids-pyrethrins (Day et al. 2017).

FAW is a pest of economic importance in its native region of the Americas. As a highly voracious and polyphagous pest, FAW became shortly after its detection in Benin a national priority challenge. Farmers' and government response was relying on the uncontrolled use of insecticides some of which are highly toxic (Ahissou et al. 2021). Thus, a need of alternative methods of protecting maize crops against FAW emerged to ensure sustainability and safety of environmental and human health. Both natural and biological control have already proved to be successful against FAW in its region of origin, basically in tropically diverse environments in Latin America. Similar environments in Benin suggest potential for the use of the biological control in the country. Earlier findings on the pest’s natural enemies and situational analyses (Houngbo et al. 2020, Dassou et al. 2021, Tepa-Yotto et al. 2022, Nagoshi et al. 2021, Aniwanou et al. 2020, Kenis et al. 2019, Agboyi et al. 2020) concurred to existing potential for biological control. Despite significant investment to increase the knowledge of determinants for sustainable management of FAW, gaps remain. In the west African context and particularly in humid forests, FAW has overlapping generations and is present year-round. However, the pest's capacity for survival of its offspring in the absence of the preferred host plant (maize) is still poorly documented. A number of parasitoids have been reported as adapting to FAW in Africa, but there is limited insight on their dynamics across seasons. Only rare empirical data are available on biological control efficacy on FAW in Africa. Addressing all these gaps is fundamental for long term management of FAW in Benin and west Africa. The current PhD programme aimed at filling the gaps through a feasibility study of FAW biological control.

5.3.2 Feasibility of biological control assessment indicators

In this thesis, the feasibility of biological control against fall armyworm was assessed using three major indicators as discussed by Barbosa and Segarra-Carmona (1993).

Pest introduction and habitat

The fall armyworm is a pest of great invasion potential. Pest introduction pathways onto the African continent are uncertain. However, genetic signatures have revealed that FAW strains present on the African continent are to date composed of more than 90% of the maize strain. The rest includes hybrids and less than 1% rice strain (Nagoshi et al. 2021, Sarr et al. 2021). Haplotypes sampled from 11 African countries indicated that Florida and the Caribbean regions are the most likely area of origin of the African infestations. In any case, the likelihood of establishment of FAW in sub-Saharan Africa was high since maize, the pest prime host plant is widely cultivated in Africa. Despite the fact that Montezano's team (2018) have comprehensively documented FAW's host plants in the continent of origin (the Americas), it is uncertain which taxa would successfully host the pest in the new environments particularly in the absence of maize crops **(Paper I and Paper II)**.

Presence and effectiveness of biocontrol agents

Three potential parasitoid species co-evolved with FAW in the Western Hemisphere i.e. the scelionid egg parasitoid *Telenomus remus* and the braconid egg-larval *Chelonus insularis* and larval *Cotesia marginiventris* parasitoid species (Molina-Ochoa et al. 2003). It was uncertain whether these species were prevalent on the African continent until it was discovered that a few taxa previously released or associated with stemborers had adapted to FAW. Therefore, these early reports of FAW parasitoids in Africa (Sisay et al. 2018 & 2019, Kenis et al. 2019) gave rise to careful reconsideration of classical biological control endeavours. Still only a couple of reports have made mention of a few parasitoid species found on FAW. Moreover, none of these explained how seasonal variations may determine species occurrence and few of them examined the wasps' effectiveness **(Paper I and Paper III)**.

Current biological control status

Evaluation of the potential for FAW biocontrol can only rely on sparse first-hand empirical data. The most common wasp recorded during field surveys (Sisay et al. 2018, 2019, Kenis et al. 2019, Agboyi et al. 2020) is the egg parasitoid *T. remus*. Field parasitism measured in a few countries provided first estimations of the potential of *T. remus* to control FAW. However, the decision to focus on augmentation or conservation instead of classical biological control using the egg parasitoid should rely on more empirical data **(Paper III)**. Moreover, the scientific basis for the magnitude to which the parasitoid should be released for cost-effective operations remains unexplored **(Paper IV)**.

5.4 Objectives and hypotheses

The thesis aimed at investigating the feasibility of biological control against *Spodoptera frugiperda* in Benin using an approach based on a set of indicators for feasibility of biocontrol from Barbosa and Segarra-Carmona (1993) and Tamo et al. (1997). This is a three-fold assessment criterion as discussed by Barbosa and Segarra-Carmona (1993) i.e. 1) pest introduction and habitat pattern; 2) presence and effectiveness of biocontrol agents; and 3) current biological control status. Specific objectives were to:

i. document the host plant range of *Spodoptera frugiperda* and the parasitoids adapting to the pest in southern and central Benin;
ii. compute the life tables of *Spodoptera frugiperda* when fed with maize and onion during the larval stage at ambient fluctuating temperatures;
iii. compare the performance of two strains of the egg parasitoid *Telenomus remus* on *Spodoptera frugiperda*;
iv. assess three release methods of the locally recruited egg parasitoid *Telenomus remus* under field cages.

The research was hypothesis-driven to address the following assumptions:

a. a variety of alternative plant species host FAW and a range of parasitoids adapt to the pest;

b. the reproduction potential of FAW on onion as an important alternative host plant in Benin supports the pest's capability to perpetuate its population in the absence of maize plants;

c. the life table statistics of two strains (local and exotic) of the most promising egg parasitoid *Telenomus remus* are not significantly different at varying prey densities and temperatures;

d. the locally recruited egg parasitoid *Telenomus remus* is equally effective over three parasitoid release methods under field cages.

5.5 Materials and methods

5.5.1 Study area

The research work was conducted in Benin. Field surveys were performed in southern and central Benin. Both laboratory and semi-field investigations were carried out at the Biorisk Management Facility (BIMAF) of the International Institute of Tropical Agriculture (IITA-Benin).

5.5.2 Fall armyworm host plants and parasitoids

Fall armyworm host plants

The sampling was completed by randomly selecting study sites in southern and central Benin during maize growing and off seasons. The sites visited included maize production farms, wet agroecosystems such as vegetable production sites, plants along streams and lowlands, plants on garbage dumps, and old maize fields. The sample size varied across locations and was based on host plant presence as the fields belonged mostly to smallholder farmers in Benin. In addition, a few wild host plants were encountered harbouring fall armyworm. Therefore, the sample number ranged from 10 to 100 plants and occasionally more on maize. Nevertheless, the sample size target was initially set to 100 plants per hectare. Any infested plant (cultivated or not) with symptoms of FAW-like damage (window panes, frass) with FAW materials (egg masses, larvae), and all suspected host plants having FAW larvae or egg masses were collected and brought to the laboratory along with the insect materials for further identification. Thereafter, the eggs were kept in Petri dishes and followed until they hatched. Collected larvae were fed with sprouting maize until pupation and kept in plastic boxes covered with muslin and perforated covers. The pupae were followed in the laboratory until emergence of the adult moths. Upon confirmation of the identity of *S. frugiperda* by morphological examination of the larvae or moths, the host plant samples were sent to taxonomists at the National Botanical Reference Center at the University of Abomey-Calavi (UAC-Benin) for identification.

Parasitoid complex

To detect the presence of parasitoids, FAW egg masses and larvae were collected from any infested plants encountered in the same sites described above during maize growing and off seasons. Suspected parasitism was monitored in the laboratory for possible parasitoid emergence. Egg masses were put in Petri dishes and checked every two days. After four days of incubation, all unhatched eggs or egg masses were kept aside and monitored for FAW egg parasitoid emergence. Larvae were fed with sprouting maize as described above, and checked regularly for parasitism. All formed parasitoid pupae were screened and conditioned in aerated Petri dishes in small Plexiglas cages until emergence. Specimens of the parasitoids that emerged from FAW eggs and larvae were conserved in 70% alcohol and shipped to the Natural History Museum in London for species-level identification.

5.5.3 Fall armyworm life tables at ambient temperatures

Experimental insect cultures of field collected FAW material were established on natural diets using the mass rearing protocol of FAW developed at the Biorisk Management Facility (BIMAF). FAW larvae were fed with sterilized sprouting maize (variety EVDT) or onion leaf (variety SAFARI) and raised to the pupal and adult stage. Larvae originating from a given host plant were maintained on the same host plant prior to experiments. The rearing conditions were 26 ± 1 °C temperature, 65 ± 5% relative humidity and 12 hours photoperiod.

FAW life table experiments were organized at ambient temperatures that fluctuated from 22 to 30 °C, on average 25.5 ± 0.03 °C. Immature stage development and survivorship was studied using 100 freshly laid individual eggs of less than 6 hours age**.** Larval survivorship was recorded daily until pupation. Newly formed pupae were transferred to another rearing box of the same size and incubated until adult emergence. Emerging adults were counted and sexed at daily observations. The adult longevity and reproduction were calculated using **pairs of** newly emerged adults. They were fed with 10% honey. A total of 30 couples from the rearing cohort previously fed with either sprouting maize or onion leaf at the larval stage were used in the experiments.

5.5.4 *Telenomus remus* phenology

Insect colonies and mass rearing

The fall armyworm egg mass used in this experiment originated from pure colonies collected from maize fields in southern Benin. They were mass reared under laboratory conditions following the protocol described above. FAW caterpillars were fed with sterilized fresh, sprouting maize (variety EVDT) and reared to pupal stage and adulthood. The emerging moths were fed with a 10% honey solution poured in capsules of cotton wool. Freshly laid eggs were collected upon female oviposition for experimental purposes.

The egg parasitoid *T. remus* was collected locally on FAW egg masses in maize fields in Athieme Assedji (6.853333°N, 1.888333°E), in southern Benin and mass reared using FAW egg masses to produce enough offspring for the experiments. The exotic strain of *T. remus* was imported from USDA-ARS CMAVE, Insect Behavior and Biocontrol Research Unit, Gainesville, Florida 32608, USA. They were kept in strict isolation facilities using local *S. frugiperda* eggs as the host for both strains of the egg parasitoid. All parasitoids (local and exotic) were reared at 26 ± 1 °C temperature, 65 ± 5% relative humidity and 12 hours photophase. To ensure that the two strains were kept strictly separated, the experiments were conducted in different rooms and separate growing chambers. The offspring of the emerging parasitoids were also kept in the growing chamber until they died.

Parasitoid life tables at constant temperatures

The experiment was a life table study of two strains of the egg parasitoid *T. remus* at 25, 28 and 31°C. Female parasitoids of the local strain were compared to the exotic strain and their performance was measured individually on a FAW egg cluster of 100 eggs glued on a cardboard (6 cm length and 2.5 cm width). Insect incubators (FITOCLIMA 1200 PLH, Aralab Rio de Muro, Portugal) were set to three separate constant experimental temperatures (25, 28 and 31°C), 65 ± 5% relative humidity and a 12-hour phase light/dark cycles. Temperature data loggers (Elitech RC 5, China) were used in the incubators to monitor temperature accuracy. Fall armyworm egg cards were prepared for the experiments by gluing the eggs with an odourless and non-toxic transparent glue. They were then exposed in glass vials (8.5 cm height and 3.0 cm diameter) to mated females of *T. remus* that were less than 12-hours old, without previous egg laying experience (Carneiro et al. 2010). One egg card of FAW was exposed to one female parasitoid per each glass vial. The vials were covered with tight micromesh tissue to avoid the parasitoid escaping and they were closed using a perforated lid for ventilation purposes. Experimental female parasitoids were fed with 10% honey solution droplets. The egg card was removed from the vials after 24 hours exposure to the parasitoid and replaced by a new egg card until the female parasitoid died. The parasitized eggs were checked for successful parasitism under a stereomicroscope (Chen et al. 2021) and kept at the same temperature until adult emergence for daily observations and measurements of development parameters and lifespan. There were 10 replicates of the female parasitoids for each strain and temperature combination, giving a total of 60 wasps used. Females that died before 24 hours post-exposure were discarded from the data analysis.

Parasitoid functional response at constant temperatures

The functional response of the egg parasitoid was computed using one individual mated female (< 12 hours age) of each of the two strains of *T. remus* without previous oviposition experience. The female was confined with fresh eggs of *Spodoptera frugiperda* (< 12 hours old) at various egg densities (10; 30; 50; 70; 100; 150 and 200) on cardboards as described by Pomari et al. (2012) at three distinct constant temperatures. All of the experiments were conducted in insect growth incubators (FITOCLIMA 1200 PLH, Aralab Rio de Muro, Portugal) at three separate constant experimental temperatures (25, 28 and 31°C), 12L:12D photophase and 65-75% relative humidity. Parasitism was measured after 24 hours of exposure of the FAW egg clusters to female wasps. Observations were done by dissection of egg masses under a stereomicroscope (Chen et al. 2021) to confirm successful parasitism. There were 10 replicates of the female parasitoids at each experimental host density for the respective temperatures, giving a total of 210 wasps tested. However, the wasps that died before the 24 hour experiment duration were discarded from the analysis.

Telenomus remus performance at various parasitoid densities

The performance of the egg parasitoid was determined at five parasitoid densities (1, 5, 10, 15, 20) of less than 12-hour old mated females without previous egg laying experience. Females of *T. remus* were tested on masses of 100-200 eggs of *S. frugiperda* at ambient temperature fluctuating from 22 to 30 °C, on average 25.5 ± 0.03 °C. Parasitoids at each density were confined with an egg mass in glass vials for 24 hours. The number of eggs in an egg mass was estimated by multiplying the number of eggs on the outer layer by the number of inner layers of each egg mass. The number of eggs on the edges was added to the total (Beserra and Parra 2005). There were two parasitoid strains (local and exotic), five parasitoid densities (1, 5, 10, 15, 20 mated females) and 10 replicates, giving a total number of 100 female parasitoids tested (Pomari et al. 2013). Total parasitism and resulting adult emergence were computed for each parasitoid density.

For this experiment, the eggs exposed to *T. remus* were followed for the first four days to remove the hatching FAW larvae, and monitored throughout the experiment to count the parasitized eggs. The male and female parasitoid offspring were counted upon emergence to calculate the sex ratio.

5.5.5 Field efficacy of *Telenomus remus*

Three parasitoid release methods were compared in a randomized complete block design with four treatments and four replicates; treatment 1) weekly parasitoid release until the maize tasselling stage (Tefera et al. 2019); treatment 2) three consecutive parasitoid releases at three day intervals until the maize tasselling stage (Cruz et al. 2018); treatment 3) parasitoid release at V4 and V10 stages of maize growth (Pomari et al. 2013); treatment 4) only FAW adults were released in control field cages and eggs on infested plants were monitored to verify cage mesh tightness and isolation from external organisms. The four treatments within blocks were separated by 20m from each other while there was 30m separation between each of the four blocks.

Rainfall in southern Benin has a bimodal pattern (Mar–Jul and Sep–Nov) which offers two distinct growing windows, the first being the major rainy season and the second the minor. All experiments were conducted at the International Institute of Tropical Agriculture (IITA-Benin) station, Cotonou, Benin (6.417500°N, 2.331500°E) during both major and minor seasons.

The artificial infestation of the plants in the experimental cages was completed using five couples of newly emerged FAW adults. The fall armyworm cohorts used in this experiment originated from larvae collected on maize fields in southern Benin. The local strain of *T. remus* used in this experiment was previously collected on FAW egg masses in maize fields in Athieme Assedji (6.853333°N, 1.888333°E) in southern Benin. Experimental clusters of fifty *T. remus*-parasitized eggs of FAW were glued on cardboard (6cm x 2.5cm) (Tefera et al., 2019) to test the three parasitoid release methods. The parasitized egg cards were hung on maize plants (one egg card per treatment) 11 days post-exposure to *T. remus* females in the laboratory, i.e. the day before expected adult parasitoid emergence. The parasitoid release commenced 7 days after emergence of the maize plants, approximately at the 4-leaf vegetative stage (V4) until tasselling. Both the moth and the natural enemies were released in the afternoons at 4:00pm. The first release of *T. remus* was done on May 30th, 2021 and on September 30th, 2021 during the major and the minor season, respectively. The last release of the parasitoid was completed on July 19th, 2021 (major season) and on November 19th, 2021 (minor season).

After the parasitoid release, all egg masses were collected every two days and three times a week from all the 15 plants within every field cage and kept separately in transparent labelled plastic bags.

The collected egg masses were conditioned separately in aerated Petri dishes, carried to the laboratory and monitored at 26 ± 1°C. These eggs were followed daily to quantify parasitism rates. The parasitized eggs were checked for successful parasitism under a stereomicroscope (Chen et al. 2021) and monitored daily until adult parasitoid emergence. No parasitism occurred on control plants and the data were not included in further analyses.

To compare the three parasitoid release methods, four parameters were considered in the data analyses for both the major and the minor seasons. These parameters include: the number of FAW eggs parasitized by *T. remus*; the number of adult parasitoids which emerged; the percentage of FAW eggs parasitized by *T. remus*; and the percentage of adult parasitoids emerged.

5.5.6 Data analysis

Fall armyworm life tables at ambient temperatures

The life table parameters of *Spodoptera frugiperda* were calculated at ambient temperatures with the aid of SAS statistical analysis tool (SAS 2013) using the program developed by Maia et al (2000). The two cohorts of experimental insects either collected from maize or onion fields and fed at the larval stage with the original host plant were analyzed separately.

Parasitoid life tables at constant temperatures

The life table parameters of *T. remus* were calculated at three constant temperatures using the SAS program developed by Maia et al. (2000). Differences in the intrinsic rate of increase values were tested for significance by estimating variances through the jackknife method (Meyer et al. 1986). The life table parameters were compared between local and exotic strains of *T. remus* by performing ANOVA using the GLM procedure in SAS followed by paired Student's t-tests (SAS 2013).

Parasitoid functional response at constant temperatures

The statistical analysis of the functional response study was completed using binary logistic regression with a logit link function to test for the effect of parasitoid strain as a factor and that of *S. frugiperda*'s egg mass density as a continuous explanatory variable on *T. remus* parasitism at different constant temperatures (Minitab 2011). The Fisher's exact tests with Bonferroni correction at the 5% level were also performed to test for significant differences among the parasitoid strains (local and exotic), followed by pairwise comparisons.

Telenomus remus performance at various parasitoid densities

The number of FAW eggs parasitized by *T. remus* were $\log_{10}(y + 1)$ transformed where necessary before analysis to meet the assumptions of normality and equal variance. Series of one-way analysis of variance (ANOVAs) followed by Tukey's post hoc tests at the 5% level and by pairwise comparisons were performed to identify significant differences (R Core Team 2012).

Field efficacy of Telenomus remus

The number of FAW egg masses parasitized by *T. remus* and of adult parasitoids emerged were log-transformed before analysis to meet the assumptions of normality and equal variance. The data were then analysed using a linear model analysis of variance (ANOVA) type II sum of squares with parasitoid release method as the fixed effect factor, and maize growth period as a categorical variable. Comparisons were done over two registration periods matching two maize growth stages: early whorl stage (0-21 days) and late whorl stage (22-49 days) (McGrath et al. 2018). All observation dates were grouped into the two periods. Tukey's post hoc tests at the 5% significance level was used to examine differences among the groups, followed by pairwise comparisons (R Core Team 2012).

The parasitism rate was calculated by dividing the total number of parasitized eggs by the total number of eggs counted within an egg mass. The percentage of emerging adult parasitoids was computed as the ratio of the total number of hatching adults to the total number of parasitized eggs. The data were subjected to angular transformation to meet the assumptions of normality and variance homogeneity. The arcsine square-root transformed data were then exposed to two-way ANOVA for testing parasitoid release method as the fixed effect factor, and maize growth period as a categorical variable. Mean separation was performed using Tukey's post hoc tests at the 5% significance level (R Core Team 2012).

5.6 Results

5.6.1 Fall armyworm host plants and parasitoids

FAW alternative host plants in southern and central Benin

The survey in southern and central Benin revealed 29 alternative host plant species of FAW, belonging to 10 families. Regardless of season, the most abundant host plant families included Poaceae, Cyperaceae and Amaranthaceae with 13, 5 and 3 species, respectively. There were ten other cultivated and nineteen other wild host species recorded. Most of the alternative cultivated host plants were recorded in Sèmè and Abomey-Calavi, which include major vegetable production sites. The highest records of wild host plants were in Abomey-Calavi, but the reason for this is unclear. We are enthusiastic to report 11 new host plant species compared to the latest records of Montezano et al (2018).

Seasonnal variation of FAW alternative host plants in southern and central Benin

FAW eggs or larvae were not found associated with all plant species across seasons. Onion (*Allium cepa* L.), Welsh onion (*Allium fistulosum* L.) and cabbage (*Brassica* *oleracea* L.) were the cultivated plant species most frequently found associated with FAW. Onion was recorded with FAW during both maize growing and off seasons, while *A. fistulosum* and *B. oleracea* were only found with FAW during off-season. FAW association with wild host plants was also season-dependent. Most of the wild host plants were recorded during off-season. The five most important wild host plant species were: *Amaranthus spinosus* L., *Cyperus roduntus* L., *Cyperus* sp., *Digitaria* cf. *horizontalis* Willdenow and *Panicum maximum* Jacquin. Only *D.* cf. *horizontalis* and *P. maximum* hosted FAW during both maize growing and off seasons. Overall FAW host plant records were higher in the 2020 off-season compared to 2018 and 2019 off-seasons.

FAW parasitoid complex in southern and central Benin

Seven parasitoid species belonging to four families were found on *S. frugiperda*. The recorded parasitoids are: *Telenomus remus* Nixon (Hymenoptera: Scelionidae), *Chelonus bifoveolatus* Szépligeti, *Coccygidium luteum* (Brullé), *Cotesia icipe* Fernandez-Triana and Fiaboe (Hymenoptera: Braconidae), *Pristomerus pallidus* (Kriechbaumer), *Charops* sp. (Hymenoptera: Ichneumonidae) and *Drino quadrizonula* (Thomson) (Diptera: Tachinidae). The collections encompass one egg parasitoid (*T. remus*), one egg-larval parasitoid (*Ch. bifoveolatus*) and the remaining five larval parasitoids representing 95; 2 and 3% of the entire parasitoid material collected, respectively. *Charops* sp. was recorded in Atlantique, Oueme, Plateau and Zou. *Chelonus bifoveolatus* was also found in the same areas and in one more department (Mono). As for *Co. luteum*, *Co. icipe*, and *P. pallidus,* they were all discovered in Atlantique, Mono, Oueme and Plateau. In addition, *Co. icipe* and *P. pallidus* were found in Zou. The egg parasitoid *T. remus* was recorded in Atlantique, Plateau and Zou while the larval parasitoid *D. quadrizonula* was identified in Atlantique only. Overall, FAW parasitoids were found in all study sites except in Kouffo, Collines and Littoral departments. Pearson's chi-squared test showed a positive correlation between the maize season and the occurrence of parasitoid species (chi-square = 188.81; df = 6; $P < 0.0001$). The parasitoids recorded during the maize growing season were far more abundant than in the absence of maize.

All collected parasitoid species were associated with FAW larvae or egg masses collected on both maize crops and wild host plant species except *D. quadrizonula* which was found in 2020 on maize only in one location of southern Benin. No *D. quadrizonula* parasitoids were found in 2018 and 2019. The egg parasitoid *T. remus* was recorded on *Panicum maximum*, while the egg-larval parasitoid *Ch. bifoveolatus* was recorded on *Bulbostylis coleotrica*, *P. maximum* and *Andropogon* sp. The larval parasitoids *Charops* sp., *Co. luteum* and *Co. icipe* were also collected on onion and wild host plant species. These larval parasitoids were collected on *D.* cf. *horizontalis*, *B. coleotrica* and *Andropogon* sp. (*Charops* sp.); D. cf. *horizontalis*, *Sorghum arundinaceum*, *Cyperus* sp. and *P. maximum* (*Co. luteum*); *A. spinosus*, *B. coleotrica* and *S. arundinaceum* (*Co. icipe*). *Pristomerus pallidus* was recorded on the wild plant species *C. roduntus* and *S. arundinaceum*.

5.6.2 Fall armyworm life tables at ambient temperatures

Stage specific development time and survivorship

Spodoptera frugiperda successfully completed its life cycle on its prime host (maize) and on its most common alternative host plant (onion). The egg-adult development time on maize was 25.76 ± 0.22 days, 9 days earlier compared to onion. Almost all eggs laid could hatch on both host plants. However, a high larval mortality was associated with onion, this resulted in only 12.24% of individuals reaching adulthood compared to maize (63.44%).

Reproduction of FAW on maize and onion

Pre-oviposition, oviposition and post-oviposition periods looked almost equal for females reared on maize and onion during their larval stage. The same trends were observed for both female and male longevity. Females reared on maize were the most prolific, more than one-thousand eggs laid. Those on onion laid 413 eggs less. The proportion of female offspring to parent reared on maize was relatively higher 0.61 vs. 0.5 on onion.

Population growth parameters of FAW on maize and onion

The net reproductive rate (R_o), the intrinsic (r_m) and finite (λ) rates of increase measured in the laboratory were relatively higher on maize (499.92; 0.27 and 1.32) compared to onion (63.77; 0.13 and 1.14), respectively. The development of FAW fed on onion at the larval stage was delayed at ambient temperatures. Females in both experiments displayed a unimodal fertility pattern with the highest number of eggs laid by females reared on maize during their larval stage. The maximum eggs laid by female *S. frugiperda* previously reared on maize occurred on day 26, one week earlier compared to females previously fed with onion during their larval stage.

5.6.3 *Telenomus remus* phenology

Parasitoid life tables at constant temperatures

Telenomus remus biological parameters were diversely affected by temperature and parasitoid strain factors. The local parasitoid strain had longer egg-adult development time (13.0 days) at 25 °C ($F_{1,212}$ = 25.9; $P < 0.0001$), shorter at 28 °C ($F_{1,135}$ = 15.2; P = 0.0001) and equal at 31 °C ($F_{1,160}$ = 3.01; P = 0.084) when compared to the exotic parasitoid strain. The higher the temperature, the shorter the egg-adult time for both the local ($F_{2,253}$ = 1317.6; $P < 0.0001$) and the exotic ($F_{2,254}$ = 743.4; $P < 0.0001$) parasitoid strains. The fastest egg-adult development time was 9.0 days for the exotic parasitoid strain at 31 °C.

Overall, the average oviposition time ranged from 5.2 (local parasitoid strain at 28 °C) to 10.8 (local parasitoid strain at 25 °C) days. There were no statistically significant differences in oviposition time between the local and the exotic parasitoid strains at any of the temperatures tested (($F_{1,26}$ = 0.80; P = 0.378), ($F_{1,25}$ = 3.19; P = 0.085), ($F_{1,25}$ = 3.85; P = 0.060) for 25; 28; and 31 °C, respectively). The lower the temperature, the longer the oviposition time for the local parasitoid strain ($F_{2,38}$ = 6.86; P = 0.002); but that was not the case for the exotic parasitoid strain which had a stable oviposition time independent of the temperature tested ($F_{2,38}$ = 1.66; P = 0.202).

The mean post-oviposition time was generally 1.0-2.7 days. This did not change significantly between the two parasitoid strains (local and exotic) at any of the constant temperatures (($F_{1,28} = 0.15$; $P = 0.696$), ($F_{1,25} = 2.02$; $P = 0.166$), ($F_{1,25} = 0.59$; $P = 0.449$) for 25; 28; and 31 °C, respectively); nor between temperatures for a given parasitoid strain, local ($F_{2,38} = 2.10$; $P = 0.135$) or exotic ($F_{2,40} = 1.63$; $P = 0.207$).

Females of both *T. remus* strains (local and exotic) generally lived for a shorter time with increasing temperature, but the same parameter (7.84-13.13 days) showed no significant differences between the two strains at any of the constant temperatures ($P > 0.05$).

The local female parasitoid strain was relatively the most prolific at 31 °C with on average 159.0 FAW eggs parasitized per female parasitoid. Female lifetime fecundity was equal between the two parasitoid strains at 25 °C ($F_{1,28} = 1.18$; $P = 0.286$), 28 °C ($F_{1,25} = 0.02$; $P = 0.884$) and 31 °C ($F_{1,25} = 0.15$; $P = 0.694$). Temperature did not affect the female exotic parasitoid strain's lifetime fecundity ($F_{2,40} = 0.05$; $P = 0.949$), nor for the local parasitoid strain, with the highest number of FAW eggs parasitized per female parasitoid at 31 °C ($F_{2,38} = 1.07$; $P = 0.355$).

Parasitoid viability (93.6-98.3 %) was not different between the two strains at any of the tested temperatures, ($F_{1,28} = 0.70$; $P = 0.406$), ($F_{1,25} = 3.76$; $P = 0.063$), ($F_{1,25} = 0.96$; $P = 0.334$) at 25; 28; and 31 °C, respectively; nor was the effect of temperature on adult emergence rate different for the two parasitoid strains, local ($F_{2,38} = 0.40$; $P = 0.668$) and exotic ($F_{2,40} = 0.64$; $P = 0.531$).

The highest female proportion in the progeny of *T. remus* (0.74) was observed on the exotic parasitoid strain. This was greater than that of the local strain at all temperatures tested (($F_{1,28} = 15.70$; $P = 0.0004$), ($F_{1,25} = 8.84$; $P = 0.006$), ($F_{1,25} = 24.54$; $P < 0.0001$) at 25; 28; and 31 °C, respectively). However, temperature did not significantly affect the sex ratio of the local ($F_{2,38} = 1.92$; $P = 0.159$) or exotic ($F_{2,40} = 1.40$; $P = 0.257$) parasitoid strains.

Parasitoid functional response at constant temperatures

The number of eggs parasitized increased while the percentage of eggs parasitized was inversely proportional to the FAW egg density, displaying type II functional response patterns. The oviposition of the exotic strain of *T. remus* was higher than that of the local strain at both 25 and 28 °C, while the local parasitoid strain was superior in warmer conditions (31 °C). In all instances, there was evidence that both parasitoid strain as a factor and the host egg density as a continuous explanatory variable were significantly different from zero for the response investigated, showing that the two variables affected *T. remus* oviposition. However, there was no interaction between these two effects.

Telenomus remus performance at various parasitoid densities

The performance of *T. remus* females (either local or exotic strain) on 100-200 egg masses exposed to different parasitoid densities (1; 5; 10; 15 and 20 wasps), varied at ambient temperature ($P < 0.05$). Differences were only occasionally observed between the two strains at the respective parasitoid densities.

The total parasitism rate was relatively higher (107.2 FAW eggs parasitized) for the local parasitoid strain at a density of 5 female wasps compared with 101.35 eggs parasitized for the exotic strain, but there were no statistically significant differences across the different parasitoid densities ($F_{4,70} = 2.13$; $P = 0.086$). Conversely, the parasitism rate was significantly lower (39.4 FAW eggs parasitized) at the 1 female wasp density of the exotic strain, 2.7-fold smaller than the highest rate ($F_{4,70} = 9.10$; $P < 0.0001$). Similar trends were recorded for all of the other parameters. In general, increased female parasitoid density beyond 5 wasps did not significantly improve the parasitoid performance on clusters of 100-200 FAW eggs. However, the sex ratio was significantly higher for the exotic female parasitoid's progeny at the highest mother density (20 wasps) ($F_{1,27} = 5.79$; $P = 0.023$).

5.6.4 Field efficacy of *Telenomus remus*

The three parasitoid release methods tested were statistically comparable in terms of the performance of *T. remus* under field cages. None of the experiments revealed significant differences between the methods in either of the cropping seasons (major and minor). However, treatment 1 (weekly releases) was the most efficient in the minor season, which was not the case during the major season where treatment 3 (release at V4 and V10) was the best. The success of the parasitoid release methods differed throughout the maize development cycle, early whorl stage (0-21 days) and late whorl stage (22-49 days). Overall, the parasitism rate improved during the late whorl stage, except that an opposite trend was observed for treatment 1 (weekly releases), during the major season. Both the parasitism and parasitoid adult emergence rates were higher during the minor season compared to the major season, 1.3 to 5.3-fold and 1.2 to 7.8-fold respectively. For all parameters measured there were significant interactions between the parasitoid release method and the maize growth period.

5.7 Discussion

5.7.1 Fall armyworm bioecology

The record of 29 alternative host plant species of FAW belonging to 10 families in southern and central Benin is the first intensive study of the FAW host plant range in Benin (**Paper I**). The current report of 29 host plants is far fewer than the list of 180 and 353 species recorded by Casmuz et al. (2010) and Montezano et al. (2018) in the Americas, respectively. We are nevertheless excited to report 11 new host plant species compared to the latest records of Montezano et al. (2018). However, our survey was limited to a two-year period, just a few years after first detection of the pest, and to selected localities in southern and central Benin. Therefore, we cannot exclude the possibility that FAW could become adapted to more host plants in the near future, and that some plant species may have been overlooked. The higher records of FAW host plants in 2020 compared to the 2018–2019 off-seasons can be a function of the natural spread of the pest. This could also be explained by the pest's ability to adapt to more host plants with time in order to expand the resource food web with increasing pest populations post detection (**Papers I and II**). However, the number of sites visited were not the same across seasons and years, as more were visited in 2020 than in previous years. The records of FAW on maize during off-seasons (2018 and 2020) were made in irrigated vegetable production areas where maize is often planted for dual purpose, i.e., diversification of food crops and edge plant. Most of the wild host plants found with FAW during off-seasons also had the pest in growing seasons (2018 and 2019).

Spodoptera frugiperda completed its life cycle on onion as one of its alternative host plants in southern and central Benin. The results showed that onion is a conducive host plant for FAW (**Papers I and II**). The extent to which an alternative host plant is conducive is an important indicator of pest population dynamics. Insect herbivore adaptation to an alternative host plant (Bale et al. 2002) allows the pest to maintain its offspring until the preferred plant is available. This is a pioneering laboratory work carried out under no-choice conditions, but previous studies emphasized the ability of FAW to complete its life cycle by crawling or ballooning using secreted silk whereby causing herbivory from crop to crop in nature (Casmuz et al., 2010; Winsou et al., 2022). Despite the poor survival rate of FAW on onion, these laboratory results support the ability of the pest to use onion as a conducive plant between maize growing seasons. The results are in agreement with previous findings (Winsou et al., 2022; Cokola et al., 2021). Nevertheless, the lower number of offspring obtained from onion might support earlier statements that onion has an antinutritional property and can be included in an IPM package against the pest (Munyore and Rioba 2020).

5.7.2 Potential for biological control

Seven parasitoid species belonging to four families were collected in this study. This report partly corroborates earlier findings in Benin by Agboyi et al. (2020) who recorded an additional egg parasitoid *Trichogramma* sp. (Hymenoptera: Trichogrammatidae) on FAW. Data analysis revealed that there is a correlation between the season (maize growing and off-season) and the occurrence of parasitoid species recorded. This may be a partial explanation of variations exhibited across different sampling efforts, while also considering that the geographic scope of those surveys are sometimes different. Moreover, the parasitoids recorded during the maize growing season were far more abundant than in the absence of maize (**Paper I**). This could be due to the fact that the host is more abundant on maize crops than during off-seasons. Similar variations have been observed on other groups of parasitoids (Segoli et al. 2020). All FAW parasitoids associated with FAW eggs and larvae collected on maize were also recorded on some of the alternative plant species surveyed, except *D. quadrizonula* that was found on maize only.

In this study, the biological parameters of *T. remus*, namely, the oviposition time, the post-oviposition time, the female longevity, the lifetime fecundity and the adult emergence rate were generally statistically comparable between local and exotic strains under all experimental temperatures (**Paper III**). However, the temperature negatively affected the egg-adult time for both local and exotic parasitoid strains. This is typical of poikilothermic organisms characterized by shortened developmental time in warmer temperatures. The results are consistent with other findings where the egg-to-adult period of *T. remus* on four *Spodoptera* species was inversely proportional to the increase in temperature (Pomari et al. 2012, Krechemer and Foerster 2015). The life table statistics in the present study closely mirror other reports (Sari et al. 2020, Oktaviani et al. 2022). There was no temperature effect on adult emergence rate of either of the experimental parasitoid strains of *T. remus*. However, regarding mass rearing for augmentative biocontrol using *T. remus* against *S. frugiperda*, previous works have shown that the highest emergence rate of the parasitoid occurred when eggs were stored at 15°C for less than 9 days (Salazar-Mendoza et al. 2020). The present investigation discovered that the local strain performed better and was actually superior to the exotic strain at higher temperatures. This concurs with the assumption that in the African context, it would be scientifically sound to introduce the exotic strain of *T. remus* in environments with cool weather only.

Higher parasitoid density did not improve the attack rate of *T. remus* for the local or exotic strains, indicating that there is a threshold beyond which it is unnecessary to utilize the addition of females for field release programs (**Papers III and IV**). This corroborates previous studies showing the same trend of parasitism stabilization despite the increase in density of *T. remus* (Salazar-Mendoza et al. 2020). The relative decline in parasitism rate with higher parasitoid density may be a result of increased competition or trade-offs on a limited food source for offspring.

Augmentative biological control using egg and egg-larval parasitoids might be the most practical approach against FAW, compared to larval parasitoids because of the cannibalistic behaviour of the pest in its larval stage (Bueno and van Lenteren 2001). Nevertheless, mass-production of parasitoids for inundative/inoculative release might not be an easy task, and it requires specialized facilities (Castellanos et al. 2019). Our observations revealed that none of the parasitoid release methods was statistically significantly superior with regard to parasitism efficiency. Treatment 1 (weekly releases), was the most demanding in terms of the number of wasps required, 1400 parasitoids released during the whole experiment, compared to the others, 600 for treatment 2 (three consecutive releases) and 400 wasps for treatment 3 (release V4 and V10). Treatment 3 (release V4 and V10) was less arduous. While the release of a large number of individuals instead of rationale deployment may provide a quick knockdown effect on the pest, our study and previous observations suggest that it is unnecessary (Winsou et al., unpublished data). Some literature suggests that release densities/dispersal arrangements of *T. remus* should be 20 to 70 release points per hectare of the egg parasitoid at a density of 100,000 to 120,000 individuals per hectare (Cruz et al. 2016, Tefera et al. 2019, Salazar-Mendoza et al. 2020). Experience with successful mass production and release of *T. remus* for more than 40 years in Venezuela gave an estimate of the cost of its production per unit of 1000 wasps in June 2021 to be USD 1.89 (Colmenarez et al. 2022). The total cost per hectare was thus USD 15.1, based on a release rate of 8000 *T. remus* individuals per hectare. Although mass production costs can appear affordable for large-scale farmers, this might not be the case for the majority of small-scale farmers in Africa. While chemical control methods are cheaper (approximately 10 US dollars per hectare), their use can be associated with adverse health and environmental effects and costs. In addition, parasitoid production requires some technical handling skills and facilities. Most African countries lack the necessary rearing capacity. This could be covered by private sector engagement, but there is not yet such an industry in West Africa. Therefore, reducing the frequency of the releases of parasitoids will reduce costs while achieving comparable results in terms of pest population reduction.

In Ghana and Niger (West Africa), some field release experiments have been conducted using the locally recruited *T. remus* against FAW (Laminou et al. 2020, Agboyi et al. 2021) but they did not investigate different release methods. In the present study, the parasitization rate of *T. remus* improved during the late whorl stage with 3 consecutive releases or release at V4 and V10 during the major season. Furthermore, there were significant interactions between the parasitoid release method and maize growth, which indicates that the effectiveness of the parasitoid release method varies across the plant phenology. In general, the attack rate and the parasitoid population increased later during the crop cycle, particularly during the minor season. There is a need to drastically curb the incidence of FAW during the early whorl stage particularly in instances of severe outbreaks when the pest can cause critical loss of the whole crop (Tepa-Yotto et al. 2021). Under severe pest pressure, the early whorl stage is the most sensitive phenological stage and requires fast-acting control interventions. This can only be achieved in an integrated pest management approach where effective biopesticides or low-toxicity chemical insecticides can complement augmentative releases without negatively affecting the activity of the parasitoids. However, maize plants have intrinsic recovery potential under pressing biotic and abiotic stresses because of their silica content (Nagaratna et al. 2022) and they can also induce defence mechanisms if they have enough rain and sufficient nutrients. Therefore, in cases of low to moderate FAW infestation, augmentative releases with *T. remus* may be an effective standalone option. Interestingly, parasitism rates were significantly higher in the early whorl stage during the major season, especially for treatment 1 (weekly releases). The findings suggest that the parasitism rate might be higher at the beginning of the major rainy season following the long drought period. The findings also suggest that combinations of parasitoid release frequencies could be explored to enhance augmentation effectiveness. As an example, weekly parasitoid releases (during the early whorl stage) can effectively complement the action of releases at V4 and V10 (during the late whorl stage).

The parasitism and parasitoid adult emergence rates were 1.3 to 5.3-fold and 1.2 to 7.8-fold higher during the minor season compared to the major season. These results are comparable to earlier findings in open fields in Ghana (Agboyi et al. 2021).

5.7.3 Identified gaps for future studies

Routine field surveys should be done to explore new host plants and parasitoids adapting to FAW. Continuous measurements in space and time will increase the knowledge on the determinants of FAW spread and field parasitism.

FAW preference was examined on separate experimental materials i.e. colonies originating from maize and onion fields. Further investigation might look at standardized material (e.g. FAW collected on maize only) and the preference on a selection of host plant species.

The success of biological control in smallholder farming systems that tend to have high synthetic insecticide use will largely depend on mindset and paradigm change and improved decision makers engagement. Although it can be judged unnecessary to import and release *T. remus*, it may still be useful to opportunistically recruit exotic breeds to complement the action of local counterparts during colder periods. However, as this was a laboratory investigation, field experiments are required to support cost-effective decisions.

Three parasitoid release methods were assessed in this study under field cages: method 1) weekly parasitoid release until the maize tasselling stage; method 2) three consecutive parasitoid releases at three-day intervals; and method 3) parasitoid release at V4 and V10 stages of maize growth. Further investigations in open fields are needed to validate the findings of statistically equal effectiveness of the three release methods.

5.7.4 Conclusions

Overall results obtained clearly met the three indicators for the feasibility of FAW biological control.

Indicator 1- Our initial assumptions that a variety of alternative plant species host FAW during maize off-season and that a wide range of local parasitoids have adapted to FAW were valid. In addition, this work illustrates that parasitoid species composition and abundance vary across seasons. We report eleven new host plant species of FAW compared to the latest records. The eleven new host plant species are: *A. cruentus, C. argentea, C. rutidosperma, B. burchellii, B. coleotricha, P. repens, C. citratus, E. pyramidalis, P. maximum, P. scrobiculatum* and *P. macrourum.*

FAW fed on onion during the larval stage could complete its development and reproduce. However, development was delayed and reproduction low on onion compared to maize.

Indicator 2- Seven parasitoid species belonging to four families were also collected during the study. *Telenomus remus* was the most abundant and frequent parasitoid species found attacking FAW. Our results are in agreement with other records of parasitoid species in the local fauna adapting to FAW elsewhere in Africa, and highlight the potential of native species in the control of FAW.

Temperatures ranging from 25 to 31 °C are suitable for the development and survival of both the local and exotic strains of *T. remus*. The same temperature range is the prevailing range in Benin. Both the local and the exotic strains of *T. remus* can be successful in Benin. Although it can be judged unnecessary to import and release *T. remus*, it may still be useful to opportunistically recruit exotic breeds to complement the action of local counterparts during colder periods. However, as this was a laboratory investigation, field experiments are required to support cost-effective decisions.

Indicator 3- This study demonstrates that minimal parasitoid release frequency can be cost-effective in the African context. The release of parasitoids at V4 and V10 stages of maize growth, proved to be as efficient as the two other methods, both of which are more demanding in terms of the number of parasitoids needed, and consequently, economic resources. However, as the current investigation was done under field cages, studies should be done in an open field situation before we can conclude that this will hold true in practice.

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Winsou, J. K., G. T. Tepa-Yotto, K. H. Thunes, R. Meadow, M. Tamò, and M.-G. Sæthre. 2022. Seasonal Variations of Spodoptera frugiperda Host Plant Diversity and Parasitoid Complex in Southern and Central Benin. Insects. 13: 491.

7 Appendix – Research Papers I – IV

Paper I

insects

MDPI

Article

Seasonal Variations of *Spodoptera frugiperda* Host Plant Diversity and Parasitoid Complex in Southern and Central Benin

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Simple Summary: The detection of fall armyworm (FAW) *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) in 2016 attacking maize fields in central and west Africa indicated the need to increase the knowledge on the pest host plants and natural enemies adapting to it. A field survey was conducted for two years (from June 2018 to January 2020) to determine host plant and parasitoid records variations across seasons (maize growing and off-seasons) in selected sites in southern and central Benin. A total of eleven new host plant species were reported for the first time, including grasses. The survey revealed seven parasitoid species belonging to four families, namely Platygastridae, Braconidae, Ichneumonidae and Tachinidae, associated with FAW on maize and alternative host plants. The most abundant parasitoid species found was the egg parasitoid *Telenomus remus* (Nixon) (Hymenoptera: Platygastridae). The study provides crucial information for understanding the bioecology of the pest and for its long-term management using natural enemies.

Abstract: Fall armyworm (FAW) *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) was recorded for the first time in 2016 attacking maize fields in central and west Africa. Soon after, several other regions and countries have reported the pest in almost the entire sub-Saharan Africa. In the present study, we assumed that (i) a variety of alternative plant species host FAW, especially during maize off-season, (ii) a wide range of local parasitoids have adapted to FAW and (iii) parasitoid species composition and abundance vary across seasons. During a two-year survey (from June 2018 to January 2020), parasitoids and alternative host plants were identified from maize and vegetable production sites, along streams and lowlands, on garbage dumps and old maize fields in southern and partly in the central part of Benin during both maize growing- and off-season. A total of eleven new host plant species were reported for the first time, including *Cymbopogon citratus* (de Candolle) Stapf (cultivated lemon grass), *Bulbostylis coleotricha* (A. Richard) Clarke and *Pennisetum macrourum* von Trinius (wild). The survey revealed seven parasitoid species belonging to four families, namely Platygastridae, Braconidae, Ichneumonidae, and Tachinidae associated with FAW on maize and alternative host plants. The most abundant parasitoid species across seasons was the egg parasitoid *Telenomus remus* (Nixon) (Hymenoptera: Platygastridae). These findings demonstrate FAW capability to be active during the maize off-season in the selected agro-ecologies and provide baseline information for classical and augmentative biocontrol efforts.

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1. Introduction

The Fall armyworm (FAW) *Spodoptera frugiperda* (J.E. Smith 1797) (Lepidoptera: Noctuidae) is one of the most economically important noctuid moth pests native to the Americas [1–3]. In Africa, the first outbreak of FAW was recorded in 2016 from central and west Africa in maize [4]. Soon after, several other regions and countries have reported the pest in almost the entire continent [5–8]. The estimated maize yield loss induced by FAW in Benin is 797.2 kg/ha, representing 49% of the commonly obtained average maize yield [9].

S. frugiperda is well known for its voracity on a range of crops and its periodical outbreaks in the Americas [1]. The larvae can feed on several cultivated or wild host plant species [1,10–12] but it is well known that maize is the prime host plant of FAW in its native range [1,6]. Despite most detections of FAW in Africa and elsewhere being chiefly on maize, it is anticipated that a range of plant species might host the pest during the maize off-season. Several of the plant families and species documented as host plants in the native range [1,12] are also common in the new invasion areas and may sustain the FAW populations in the absence of maize crops.

Sustainable FAW management strategies cannot be designed in areas of recent invasion without a prior in-depth assessment of the indigenous parasitoid biodiversity and related biological control potential. In extensive inventories in the Americas and the Caribbean Basin, more than 150 parasitoid species were found to be associated with FAW [13]. Among these, *Telenomus remus* (Nixon, 1937) (Hymenoptera: Platygastridae) was recognized as the most relevant naturally occurring egg parasitoid species. Initial inventory of the local fauna in Africa led to the discovery of *T. remus* [14] and other parasitoid species adapting to FAW, including *Chelonus bifoveolatus* Szépligeti, *Charops* sp., *Cotesia icipe* Fernandez-Triana and Fiaboe and *Coccygidium luteum* Brullé [15,16], but little is known about their seasonal variations. Therefore, the present study aimed at investigating in detail the seasonal variations of the host range and parasitoid diversity of FAW in Benin based on three assumptions (i) a variety of alternative plant species host FAW especially during maize off-season, (ii) a range of local egg and larval parasitoids have adapted to FAW and (iii) parasitoid species composition and abundance vary across seasons.

2. Materials and Methods

2.1. Study Sites

The study area is southern Benin and a few locations in the central parts of the country (Figure 1). The climate of southern Benin is characterized by two dry seasons (from December to February and August) and two rainy seasons (from March to July and September to November), respectively. In the central part of the country, there is one main rainy season from June to September and one dry season from October to May. The rainy season and the dry season match with maize's growing and off-seasons, respectively. The survey sites were in all southern Benin departments, namely Atlantique, Kouffo, Mono, Littoral, Oueme, Zou and Plateau, but restricted to Collines department in central Benin. In total, 40 localities (year 2018), 23 localities (year 2019) and 25 localities (year 2020) were surveyed for both FAW host plant range and parasitoid complex records (Figure 1).

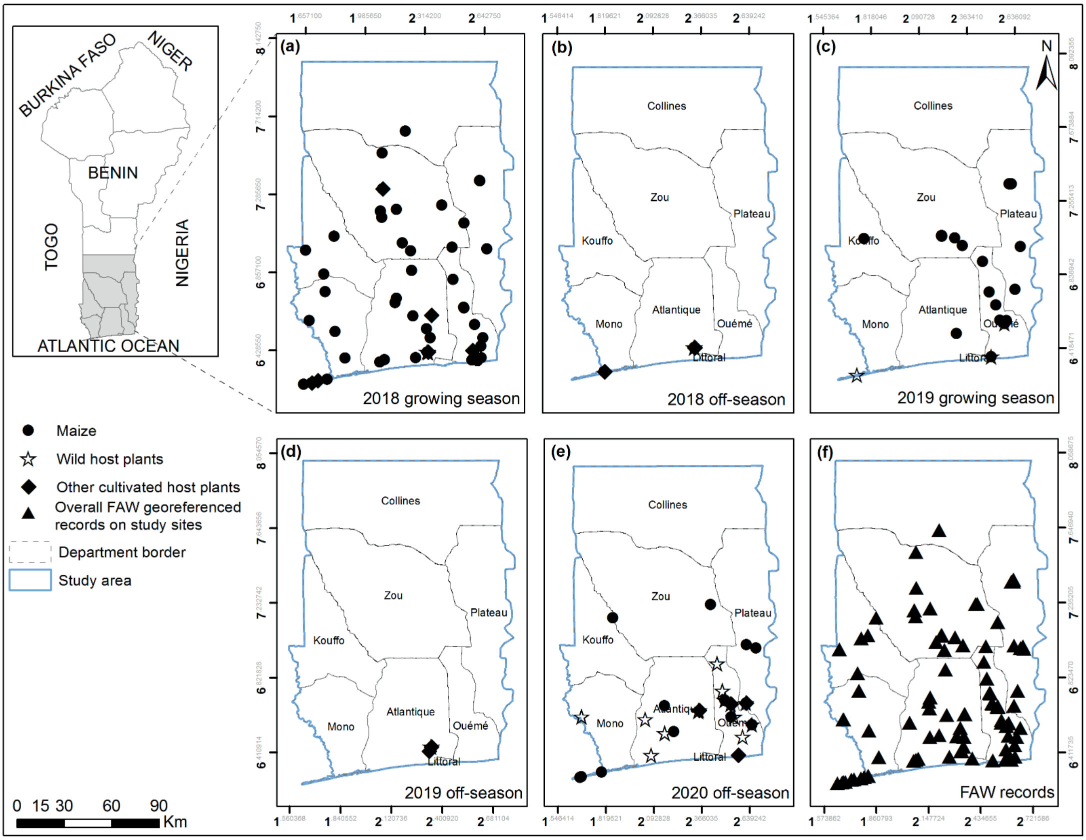

Figure 1. FAW occurrences on maize and alternative host plants during (**a**,**c**) growing seasons and (**b**,**d**,**e**) off-seasons for the years 2018, 2019 and 2020, and (**f**) overall FAW georeferenced records on study sites in southern and central Benin.

2.2. Field Surveys

Maize production sites and wet agroecosystems such as vegetable production sites, plants along streams and lowlands, plants on garbage dumps and old maize fields were selected randomly for sampling in southern and partly in central Benin during maize growing and off-season. Any infested plant (cultivated or not) with symptoms of FAW-like damage (window panes, frass) and all suspected host plants having FAW larvae or egg-masses were collected and brought to the laboratory for further identification. The sample size target was set to 100 plants to be sampled per hectare. However, because most fields visited belonged to smallholder farmers, it was uncommon to find big farms. In addition, few wild host plants were encountered harboring fall armyworm. Therefore, the sample size was based on host plant presence and ranged from 10 to 100 plants and occasionally more on maize fields. The eggs were kept in Petri dishes and followed until they hatched. Larvae were kept in plastic boxes (4.2 cm diameter; 5.3 cm height) covered with muslin and perforated cover, and fed with sprouting maize until pupation. The pupae were kept in the same type of plastic boxes until emergence of the adult moths. Upon confirmation of the identity of *S. frugiperda* by morphological examination of the larvae or moths, the host plant samples were sent to taxonomists at the National Botanical Reference Center at the University of Abomey-Calavi (UAC-Benin) for identification. All FAW host plants' georeferenced points are provided in File S1.

To detect the presence of parasitoids, FAW larvae and egg-masses were collected from any of the infested plants as described above and monitored in the lab. Egg-masses were put in Petri dishes and checked at two-day intervals in order to record any larval emergence. After four days of incubation, all unhatched eggs or egg-masses were kept aside and monitored for FAW egg parasitoid emergence. Larvae were fed with sprouting maize as described above, and checked regularly for any signs of parasitism. Parasitoid pupae were transferred to small cages until emergence. Specimens of the parasitoids collected on FAW eggs and larvae were shipped to the Natural History Museum in London for species-level identification. All FAW parasitoids' presence records are available in Files S2–S8.

2.3. Data Analysis

The correlation between the occurrence of parasitoid species and seasonal variations was tested by performing the chi-squared test for association (Ho < 0.05; H1 > 0.05) using R version 1.3.1093.

3. Results

3.1. FAW Host Plants Range

The survey in southern and central Benin revealed 29 alternative host plant species of FAW, belonging to 10 families. We are excited to report 11 new host plant species compared to the latest records of Montezano et al. [12] (Table 1). Regardless of season, the most abundant host plant families included Poaceae, Cyperaceae and Amaranthaceae with 13, 5 and 3 species, respectively. Ten and nineteen of the records comprised other cultivated and wild host species, respectively (Table 1). Most of the alternative cultivated host plants were recorded in Sèmè and Abomey-Calavi (Table 1), which harbor major vegetable production sites. The highest records of wild host plants were in Abomey-Calavi, but the reason for this is unclear (Table 1).

Table 1. FAW host plant species recorded in southern and central Benin. The eleven new host plant species records are in bold.

Host Plant Type	Family Name	Scientific Name	Sites of Collection
Cultivated	Amaranthaceae	***Amaranthus cruentus* L.** ***Celosia argentea* L.**	Sèmè Azowlissè
	Amaryllidaceae	*Allium cepa* L. *Allium fistulosum* L.	Abomey-Calavi, Agoué, Grand-Popo, Sèmè, Zinvié Abomey-Calavi
	Asteraceae	*Lactuca sativa* L.	Sèmè
	Brassicaceae	*Brassica oleracea* L.	Abomey-Calavi
	Malvaceae	*Gossypium hirsutum* L.	Dan
	Poaceae	***Cymbopogon citratus* (de Candolle) Stapf** *Saccharum officinarum* L. *Sorghum bicolor* (L.) Mönch	Sèmè Zinvié Sakété
Wild	Aizoaceae	*Trianthema portulacastrum* L.	Sèmè
	Amaranthaceae	*Amaranthus spinosus* L.	Grand-Popo, Sèmè
	Cleomaceae	***Cleome rutidosperma de Candolle***	Azowlissè
	Cyperacea	***Bulbostylis burchellii* (Ficalho and Hiern) C.B. Clarke**	Abomey-Calavi

Table 1. *Cont.*

Host Plant Type	Family Name	Scientific Name	Sites of Collection
	Cyperacea	***Bulbostylis coleotricha* (A. Richard) Clarke**	Abomey-Calavi
		Cyperus rotundus L.	Abomey-Calavi
		Cyperus retrorsus Chapman	Abomey-Calavi
		Cyperus sp.	Adjohoun, Tori-Avamè
	Euphorbiaceae	*Ricinus communis* L.	Zinvié
	Poaceae	*Andropogon* sp.	Adjohoun, Athiémé
		Digitaria sp. cf. *horizontalis* Willdenow	Abomey-Calavi
		***Echinochloa pyramidalis* (Lamarck) Hitchcock and Chase**	Zinvié, Kpomassè
		Eleusine indica (L.) Grtner	Tori-Avamè
		***Panicum maximum* Jacquin**	Abomey-Calavi, Zinvié, Misserete, Bonou, Dangbo, Adjohoun
		***Panicum repens* L.**	Abomey-Calavi
		***Paspalum scrobiculatum* L.**	Abomey-Calavi
		***Pennisetum macrourum* von Trinius**	Adjohoun, Azowlissè
		Sorghum arundinaceum (Desvaux.) Stapf.	Aguégués, Athiémé, Avrankou, Tori-Avamè, Ouidah, Bonou
		Rottboellia cochinchinensis (Loureiro) W.D.Clayton	Sakété, Athiémé

Not all plant species were found associated with FAW eggs or larvae across seasons (Figures 1 and 2). Our findings revealed that the numbers of recorded alternative FAW host plants whether cultivated or wild increased during the observation period (Figure 1).

Onion (*Allium cepa* L.), Welsh onion (*Allium fistulosum* L.) and cabbage (*Brassica oleracea* L.) were the cultivated plant species most frequently found associated with FAW (Figure 2a). Onion was recorded with FAW during both maize growing and off-season, while *A. fistulosum* and *B. oleracea* were only found with FAW during off-season. FAW association with wild host plants was also season-dependent. Most of the wild host plants were recorded during off-season (Figure 2b). The five most important wild host plant species were: *Amaranthus spinosus* L., *Cyperus roduntus* L., *Cyperus* sp., *Digitaria* cf. *horizontalis* Willdenow and *Panicum maximum* Jacquin. Only *D.* cf. *horizontalis* and *P. maximum* hosted FAW during both maize growing and off-season. Overall FAW host plant records were higher in 2020 off-season compared to 2018 and 2019 off-seasons (Figure 1). FAW was found associated with maize during 2018 and 2020 off-seasons. Conversely, wild host plants were recorded in both 2018 and 2019 growing seasons.

3.2. FAW Parasitoid Complex

Seven parasitoid species belonging to four families were collected: *Telenomus remus* Nixon (Hymenoptera; Platygastridae), *Chelonus bifoveolatus* Szépligeti, *Coccygidium luteum* (Brullé), *Cotesia icipe* Fernandez-Triana and Fiaboe (Hymenoptera; Braconidae), *Pristomerus pallidus* (Kriechbaumer), *Charops* sp. (Hymenoptera; Ichneumonidae) and *Drino quadrizonula* (Thomson) (Diptera; Tachinidae) (Table 2). The collections encompassed one egg parasitoid (*T. remus*), one egg-larval parasitoid (*Ch. bifoveolatus*) and the remaining five larval parasitoids representing 95; 2 and 3% of the entire parasitoid material collected, respectively (Figure 3).

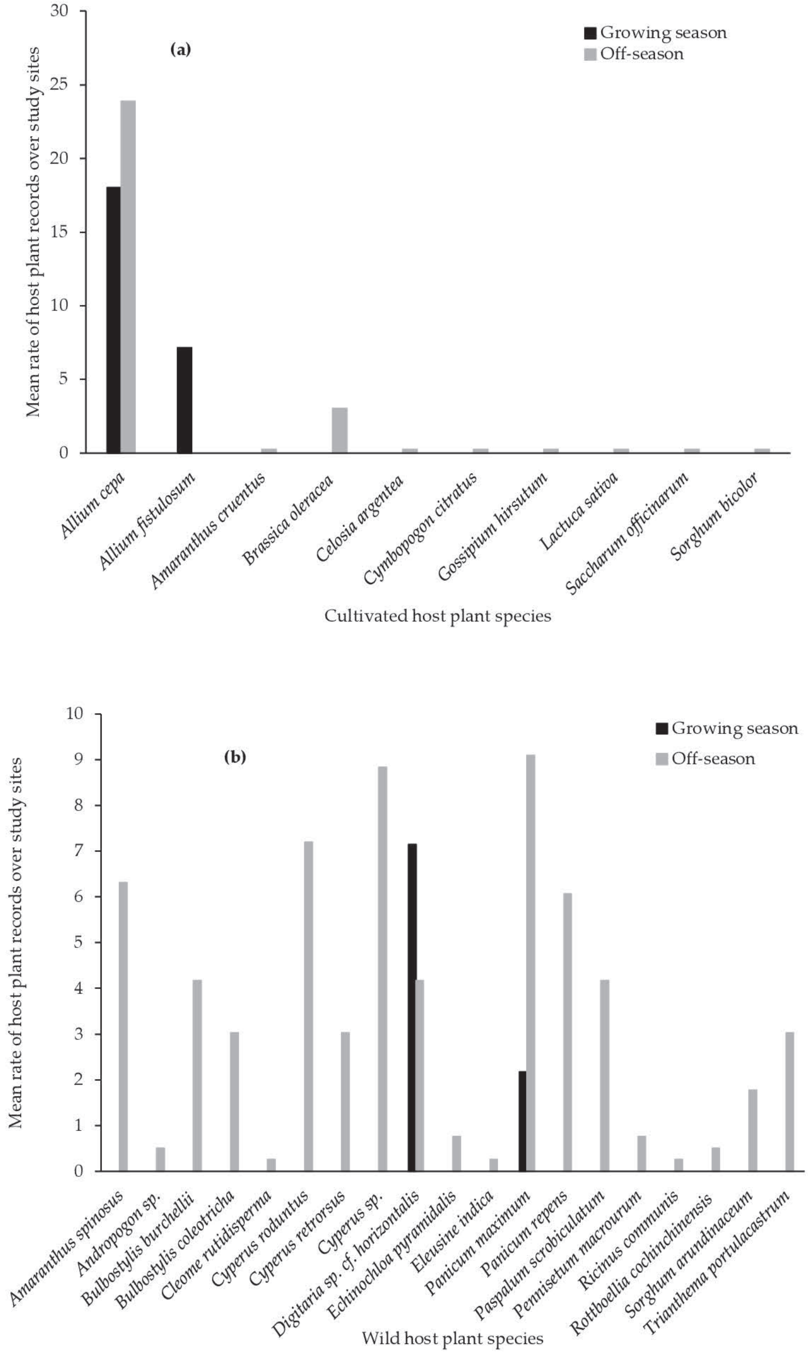

Figure 2. FAW host plant (**a**) cultivated crop and (**b**) wild species records over all study sites. The rate of host plant records for each study site is obtained by dividing the number of plants infested with FAW by the number of sites surveyed.

Charops sp (Figure 4) was recorded in Atlantique, Oueme, Plateau and Zou. *Chelonus bifoveolatus* (Figure 5) was also found in the same areas and in one more department (Mono). As for *Co. luteum* (Figure 6), *Co. icipe* (Figure 7) and *P. pallidus* (Figure 8), they were all discovered in Atlantique, Mono, Oueme and Plateau. In addition, *Co. icipe* and *P. pallidus* were found in Zou. The egg parasitoid *T. remus* (Figure 9) was recorded in Atlantique, Plateau and Zou while the larval parasitoid *D. quadrizonula* was identified in Atlantique only. Overall, FAW parasitoids were found in all study sites except in Kouffo, Collines and Littoral departments. Pearson's chi-squared test showed a positive correlation between the maize season and the occurrence of parasitoid species (chi-square = 188.81; df = 6; $p = 2.2 \times 10^{-16}$).

Table 2. FAW parasitoids complex associated with alternative host plants and maize in southern and central Benin.

Order, Family and Species	Locality	Host Plant	FAW Stage Collected
Hymenoptera: Platygastridae *Telenomus remus* Nixon	Ab-Cal; Aid (Ket); Zinv; Ouin; Zog	Maize; *Panicum maximum*	Egg
Hymenoptera: Braconidae *Chelonus bifoveolatus* Szépligeti	GP; Ag; Ket; Aid (Ket); Glo; Ab-Cal; Adj; Kpa; Pob; Zog; Avr; Zag; Azo	Maize; *Bulbostylis coleotrica*; *Panicum maximum*; *Andropogon* sp.	Larva; Egg
Coccygidium luteum (Brullé)	Ket; Ab-Cal; Bon; Adj; Azo; GP	Maize; *Digitaria* cf. *horizontalis; Allium cepa; Sorghum arundinaceum; Cyperus* sp.; *Panicum maximum*	Larva
Cotesia icipe Fernandez-Triana and Fiaboe	Glo; Mas; GP; Dra; Kpa; Sak; Mis; Ab-Cal; Zog; Zag; Ath	Maize; *Amaranthus spinosus*; *Bulbostylis coleotrica*; *Allium cepa*; *Sorghum arundinaceum*	Larva
Hymenoptera: Ichneumonidae *Charops* sp.	Glod; Mas; Pob; Ab-Cal; Dan; Adj; Mis; Pob; Azo; Ak-Mis; Ouin; Zog; Ab-Cal; Azo; Zag; All; Dan; Adja; Adj	Maize; *Digitaria* sp.; *Allium cepa; Bulbostylis coleotrica; Andropogon* sp.	Larva
Pristomerus pallidus (Kriechbaumer)	Ket; Hou (Ag); Ab-Cal; Kpa; Zog; Ath; Avr	Maize; *Cyperus roduntus*; *Sorghum arundinaceum*	Larva
Diptera: Tachinidae *Drino quadrizonula* (Thomson)	Ab-Cal	Maize	Larva

Aidjedo (Ketou): Aid (Ket); Abomey-Calavi: Ab-Cal; Zinvié: Zinv; Ouinhi: Ouin; Zogbodomey: Zog; Glo: Glo; Adjohoun: Adj; Kpankoun: Kpa; Pobè: Pob; Avrankou: Avr; Zagnanado: Zag; Grand-Popo: GP; Agoué: Ag; Ketou: Ket; Azowlissè: Azo; Bonou: Bon; Sakete: Sak; Massi: Mas; Drabo: Dra; Missérété: Mis; Athiémé: Ath; Glodjigbé: Glod; Dangbo: Dan; Akpro-Missérété: Ak-Mis; Allada: All; Adjawèrè: Adja; Houègbo (Agon): Hou (Ag).

All collected parasitoid species were associated with FAW larvae or egg-masses collected on both maize crops and wild host plant species (Table 3) except *D. quadrizonula* which was found in 2020 on maize only (Table 2) in one location of southern Benin. No *D. quadrizonula* parasitoids were found in 2018 and 2019. The egg parasitoid *T. remus* was recorded on *Panicum. maximum*, while the egg-larval parasitoid *Ch. bifoveolatus* was recorded on *Bulbostylis coleotrica*, *P. maximum* and *Andropogon* sp. The larval parasitoids *Charops* sp., *Co. luteum* and *Co. icipe* were also collected on onion and wild host plant species. These larval parasitoids were collected on *D.* cf. *horizontalis*, *B. coleotrica* and *Andropogon* sp. (*Charops* sp.); *D.* cf. *horizontalis*, *Sorghum arundinaceum*, *Cyperus* sp. and *P. maximum* (*Co. luteum*); *A. spinosus*, *B coleotrica* and *S. arundinaceum* (*Co. icipe*). *Pristomerus pallidus* was recorded on the wild plant species *C. roduntus* and *S. arundinaceum*.

Table 3. FAW parasitoids recorded on the pest eggs and larvae collected on alternative host plants.

Order, Family and Species	**Alternatives Host Plants**	**Number of Collected Specimens**
Hymenoptera: Platygastridae *Telenomus remus*	*Panicum maximum*	100
Hymenoptera: Braconidae *Chelonus bifoveolatus*		
	Bulbostylis coleotrica	1
	Panicum maximum	2
	Andropogon sp.	1
Coccygidium luteum	*Digitaria* cf. *horizontalis*	1
	Allium cepa	1
	Sorghum arundinaceum	1
	Cyperus sp.	1
	Panicum maximum	2
Cotesia icipe	*Amaranthus spinosus*	1
	Bulbostylis coleotrica	4
	Allium cepa	1
	Sorghum arundinaceum	1
Hymenoptera: Ichneumonidae	*Digitaria* sp.	1
Charops sp.	*Allium cepa*	3
	Bulbostylis coleotrica	3
	Andropogon sp.	3
Pristomerus pallidus	*Cyperus roduntus*	1
	Sorghum arundinaceum	1

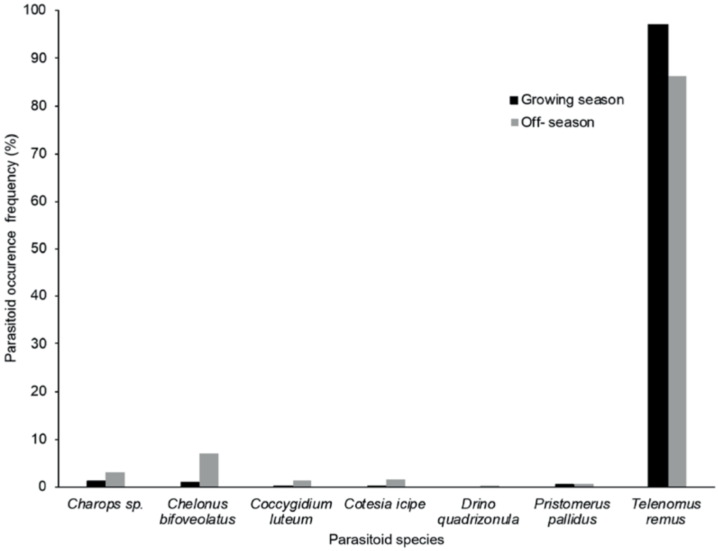

Figure 3. Occurrence of parasitoid species recorded in maize growing and off-seasons in southern and central Benin. The percentage of parasitoid frequency was calculated by dividing the number of the parasitoid species records by the total records of all parasitoid species.

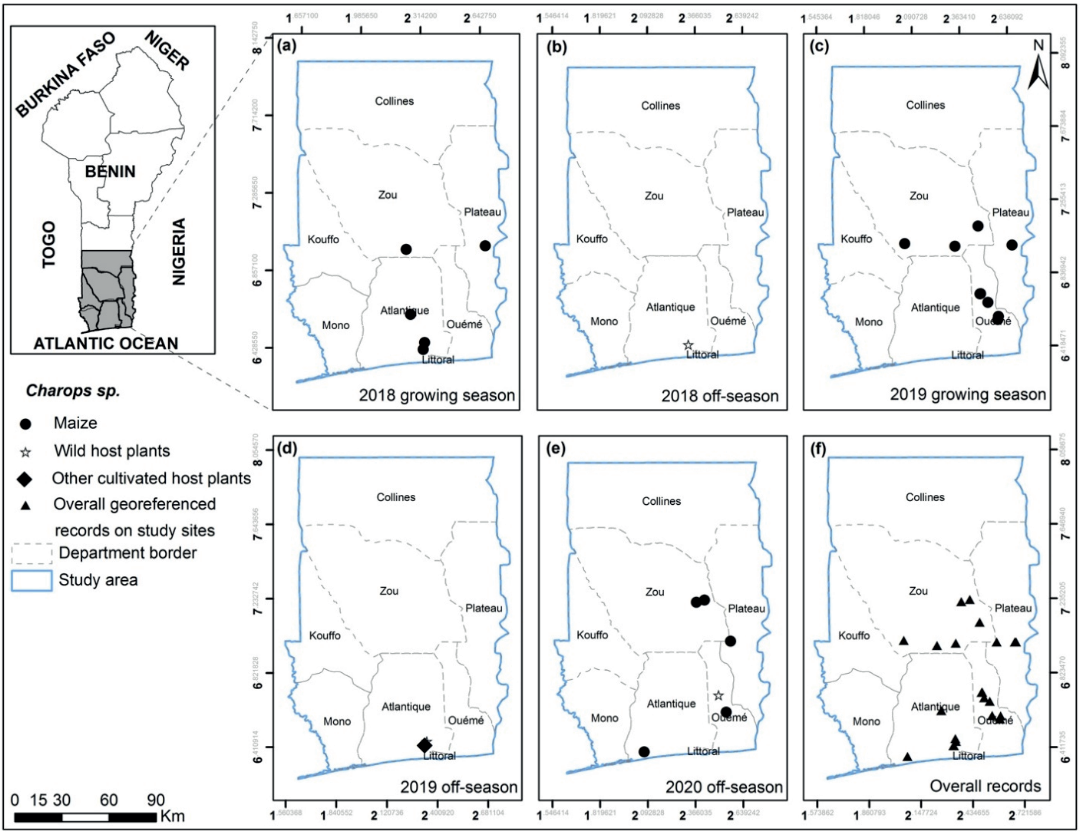

Figure 4. *Charops* sp. georeferenced records on maize, wild and other cultivated host plants for the years 2018, 2019 and 2020 on the study sites for growing (**a**,**c**) and off-seasons (**b**,**d**,**e**) in southern and central Benin (**f**).

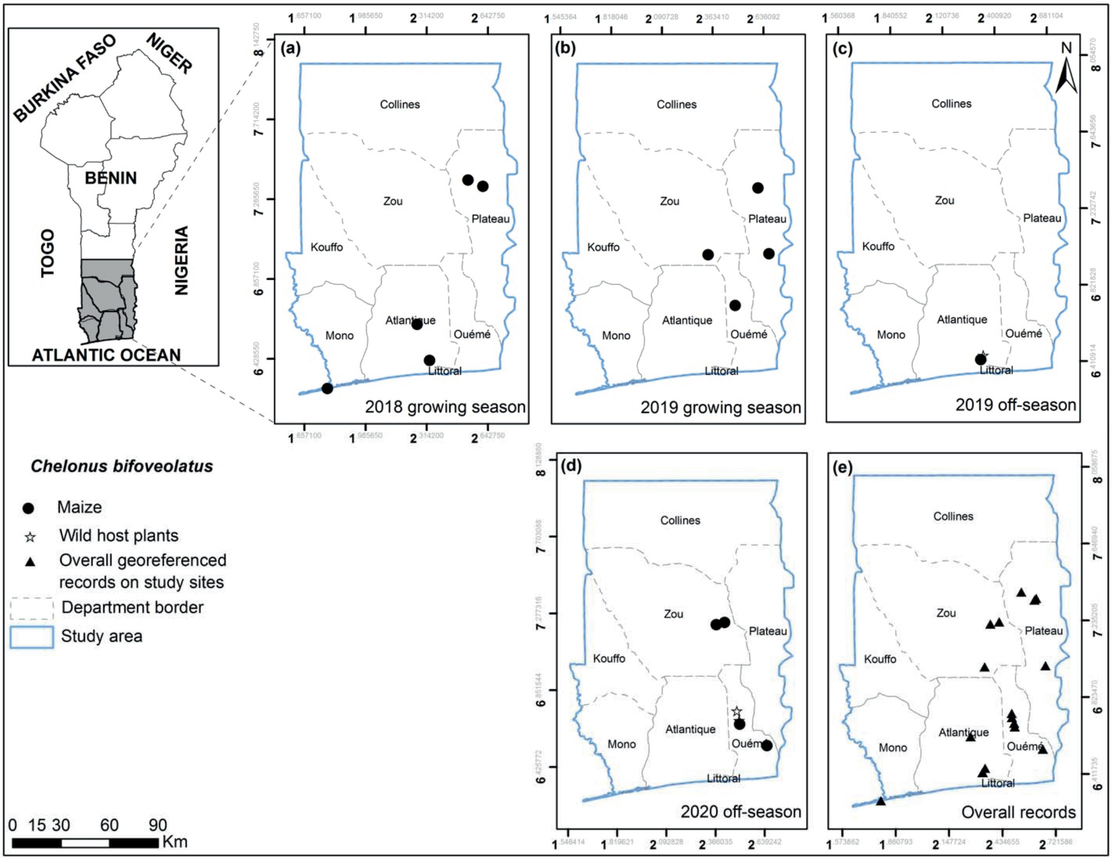

Figure 5. *Chelonus bifoveolatus* georeferenced records on maize, wild and other cultivated host plants for the years 2018, 2019 and 2020 on the study sites for growing (**a**,**b**) and off-seasons (**c**,**d**) in southern and central Benin (**e**). No *C. bifoveolatus* were found in the 2018 off-season.

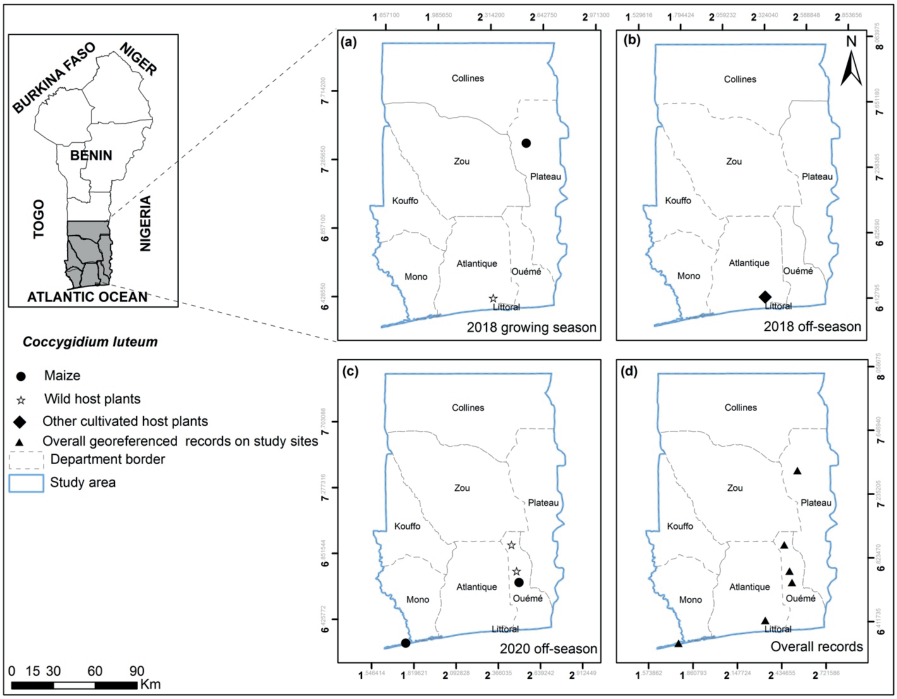

Figure 6. *Coccygidium luteum* georeferenced records on maize, wild and other cultivated host plants for the years 2018 and 2020 on the study sites for growing (**a**) and off-seasons (**b**,**c**) in southern and central Benin (**d**). No *C. luteum* were found in 2019.

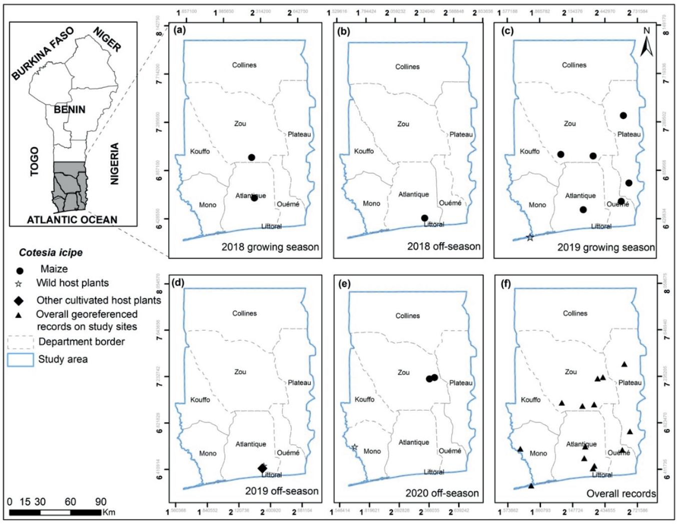

Figure 7. *Cotesia icipe* georeferenced records on maize, wild and other cultivated host plants for the years 2018, 2019 and 2020 on the study sites for growing (**a**,**c**) and off-seasons (**b**,**d**,**e**) in southern and central Benin (**f**).

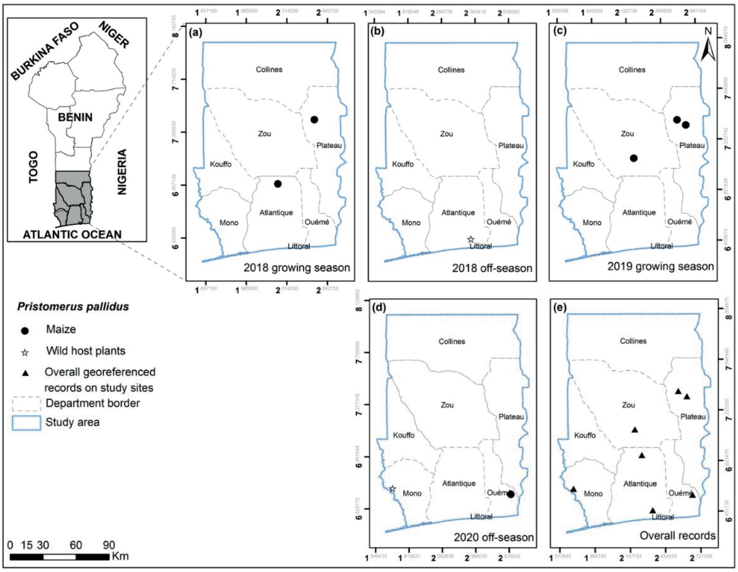

Figure 8. *Pristomerus pallidus* georeferenced records on maize, wild and other cultivated host plants for the years 2018, 2019 and 2020 on the study sites for growing (**a**,**c**) and off-seasons (**b**,**d**) in southern and central Benin (**e**). No *P. pallidus* were found in the 2019 off-season.

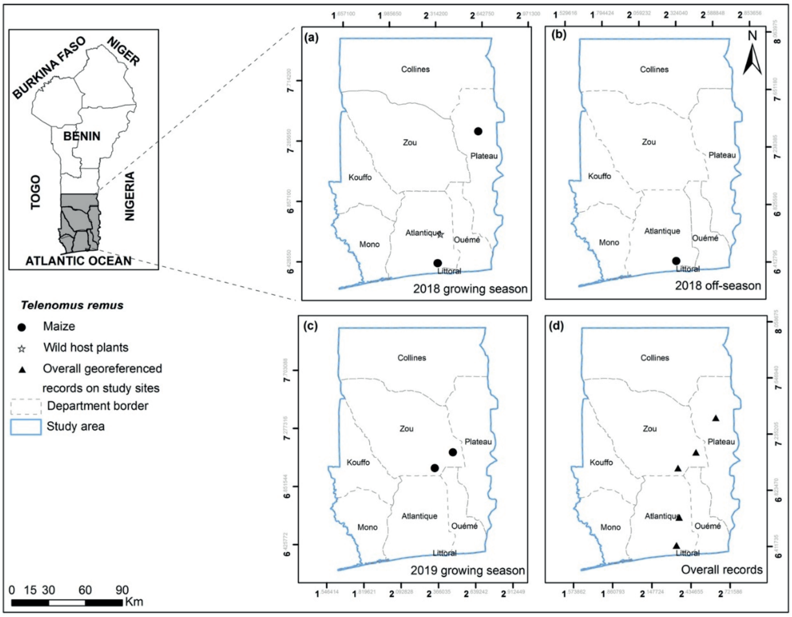

Figure 9. *Telenomus remus* georeferenced records on maize, wild and other cultivated host plants for the years 2018 and 2019 on the study sites for growing (**a**,**c**) and off-seasons (**b**) in southern and central Benin (**d**). No *T. remus* were found in the 2019 or 2020 off-season.

4. Discussion

4.1. FAW Host Plant Range

The record of 29 alternative host plant species of FAW belonging to 10 families in southern and central Benin is the first intensive study of the FAW host plant range in Benin. The current report of 29 host plants is far fewer than the list of 180 and 353 species recorded by Casmuz et al. [1] and Montezano et al. [12] in the Americas, respectively. We are nevertheless excited to report 11 new host plant species compared to the latest records of Montezano et al. [12] (Table 1). However, our survey was limited to a two-year period, just a few years after first detection of the pest, and to selected localities in southern and central Benin. Therefore, we cannot exclude the possibility that FAW could become adapted to more host plants in the near future, and that some plant species may have been overlooked. The higher records of FAW host plants in 2020 compared to the 2018–2019 off-seasons can be a function of the natural spread of the pest. This could also be explained by the pest ability to adapt to more host plants with time in order to expand the resource food web with increasing pest populations post detection. However, the numbers of sites visited were not the same across seasons and years, there were higher in 2020 than in previous years. The records of FAW on maize during off-seasons (2018 and 2020) occurred in irrigated vegetable production areas where maize is often planted for dual purpose, i.e., diversification of food crops and fence plant. Most of the wild host plants found with FAW during off-seasons also had the pest in growing seasons (2018 and 2019).

Our study demonstrates that a variety of alternative plant species host FAW during maize off-season and explains why important pest infestation levels are commonly observed on maize crops after long off-season periods. The results are in agreement with earlier reports that FAW has a flexible host plant range which plays an important role in the long-term evolutionary survival of the pest [17]. The present corroborates previous observations that FAW, without a diapause mechanism, has developed survival strategy by feeding and maintaining its populations on alternative host plants [1]. FAW sustains its offspring on cultivated and wild alternative host plants (a high number of grasses) until the next maize growing season which clearly has implications for pest management.

The potential of grasses as oviposition sites for FAW supports the theory that *S. frugiperda* prefers C4 plants including maize as opposed to C3 plants [18,19]. This might be explained by the nutritional quality of C4 plants, which best fits the needs of the pest, compared to hosts from other botanical families [17,20]. Nevertheless, the question remains whether FAW is able to complete its whole life cycle on plants such as grasses that are weeds or that grow randomly. It is well known that FAW is highly voracious with the potential to attack different plant organs and then become either defoliator, cutter, granivore or borer [21]. FAW has the ability to move to nearby plants by crawling or by ballooning through secreted silks [1]. Therefore, the pest could complete its life cycle by moving to grasses.

4.2. FAW Parasitoid Complex

Seven parasitoid species belonging to four families were collected in this study. This report partly corroborates earlier findings in Benin by Agboyi et al. [15] who recorded an additional egg parasitoid *Trichogramma* sp. (Hymenoptera: Trichogrammatidae) on FAW. Data analysis revealed that there is a correlation between the season (maize growing and off-season) and the occurrence of parasitoid species recorded (Table 3). This may be a partial explanation of variations exhibited across different sampling efforts, while also considering that the geographic scope of those surveys are sometimes different. Moreover, the parasitoids recorded during the maize growing season were far more abundant than in the absence of maize. This could be due to the fact that the host is more abundant on maize crops than during off-seasons. Similar variations have been observed on other group of parasitoids [22].

All FAW parasitoids associated with FAW eggs and larvae collected on maize were also recorded on some of the alternative plant species surveyed except *D. quadrizonula* found on maize only.

Our results support model predictions on the capability of FAW and parasitoids to survive on most of the habitats in the area already heavily invaded by FAW and those potentially at risk [23]. The findings suggest some similarities in the plant volatiles induced by FAW damage on the prime host plant and those induced on alternative host plants. The plant volatiles emitted by FAW alternative host plants may play an important role for the parasitoids, possibly as attractant cues for the parasitism of FAW [24].

This work and earlier investigations in the Americas and especially in the Caribbean basin [13,25] confirm that in the absence of maize several host plants, either cultivated or wild, can constitute a reservoir of a range of FAW parasitoids. Nevertheless, the proportion of collected parasitoids is by far higher on maize (97% in this study) than all other alternative hosts combined, similar to earlier observations [13]. A possible explanation for this occurrence is that maize, being the preferred plant species supports higher populations of the host insect (FAW), also attracts more FAW parasitoids. Large numbers of *T. remus* were collected throughout the survey which concurs to the great potential of the species for biological control programs against FAW [14,15,23].

It is noteworthy to mention that our results are in agreement with recent records of parasitoid species in the local fauna adapting to FAW [15]. However, because of the limited geographical scope of our study, we cannot exclude the possibility that other species might have been overlooked. Nonetheless, the present report shows that further biocontrol efforts in Africa should carefully consider the potential of locally available parasitoid species along with the introduction of exotic species from the area of origin of FAW in the Americas.

5. Conclusions

Our initial assumptions that a variety of alternative plant species host FAW during maize off-season and that a wide range of local parasitoids have adapted to FAW were valid. In addition, this work illustrates that parasitoid species composition and abundance vary across seasons. We report eleven new host plant species of FAW compared to the latest records. The eleven new host plant species are: *A. cruentus, C. argentea, C. rutidosperma, B. burchellii, B. coleotricha, P. repens, C. citratus, E. pyramidalis, P. maximum, P. scrobiculatum* and *P. macrourum.* Seven parasitoid species belonging to four families were also collected during the study. *T. remus* was the most abundant and frequent parasitoid species found attacking FAW. Our results are in agreement with other records of parasitoid species in the local fauna adapting to FAW elsewhere in Africa, and highlight the potential of native species in the control of FAW.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/insects13060491/s1, File S1: FAW host plant records; File S2: *Charops* sp. records; File S3: *Chelonus bifoveolatus* records; File S4: *Coccygidium luteum* records; File S5: *Cotesia icipe* records; File S6: *Drino quadrizonula* records; File S7: *Pristomerus pallidus* records; File S8: *Telenomus remus* records.

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Paper II

Life history parameters of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) on two different host plants at ambient temperatures: implications on population dynamics during maize off-seasons in southern Benin (West Africa)

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Abstract

The fall armyworm (FAW) *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae) is an invasive pest in Africa of great importance. Aside from its prime host plant (maize), FAW was frequently recorded in association with onion (*Allium cepa* L.) during the maize off-season in southern and central Benin. However, to date there is no available information on the capacity of FAW to complete its life cycle on onion. This work aimed at filling that gap by calculating the life history parameters of FAW at ambient temperatures. The temperatures fluctuated from 22 to 30 °C, on average 25.5 ± 0.03 °C. Two different host plants were tested separately i.e., larvae were fed with maize (preferred) or onion (alternative) as host plant species. We assumed that the reproduction potential of FAW on onion, which was frequently found associated with FAW eggs and larvae in Benin, could explain the pest capability to perpetuate its population in the absence of maize plants. *S. frugiperda* successfully completed its life cycle on its prime host (maize) and on its most common alternative host plant (onion). The egg-adult development time on maize was 25.76 ± 0.22 days, 9 days earlier than on onion. Females reared on maize oviposited 1267.46 ± 88.16 eggs each on average while those reared on onion laid 854.36 ± 102.75 eggs. The proportion of female offspring to parents reared on maize was relatively higher with 0.61 vs. 0.5 on onion. The net reproductive rate (R_o), the intrinsic (r_m) and finite (λ) rates of increase were higher on maize (499.92; 0.27; 1.32) compared to onion (63.77; 0.13; 1.14), respectively.

Keywords: fall armyworm, alternative host plant, maize off season, life table.

Introduction

The fall armyworm (FAW) *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae) is an invasive pest of global importance. FAW is native to tropical and subtropical environments of the Americas where it is a major pest of maize (*Zea mays*) L. (Cyperales: Poaceae) with a wide range of alternative host plants (Pogue 2002, Day et al. 2017, Montezano et al. 2018, FAO 2021). FAW was first recorded in early 2016 in the west and central African countries of Nigeria, Benin, Togo and São Tomé and Principe (Goergen et al. 2016). Since then, it has been reported in almost all sub-Saharan Africa causing significant damage to maize crops (FAO 2022), becoming a major agricultural biorisk with a high potential to threaten household income and food security of farming communities (Day et al. 2017, Houngbo et al. 2020, Eschen et al. 2021, FAO 2021).

Previous sampling efforts showed that FAW eggs and larvae were frequently recorded in association with onion, *Allium cepa* L. (Amaryllidaceae: Asparagales) in Alto Paranaíba, Minas Gerais, Brazil (Fernandes et al. 2012), in South Kivu, eastern Democratic Republic of Congo (Cokola et al. 2021) and in southern and central Benin (Winsou et al. 2022). Onion is a worldwide culinary and therapeutic food plant which is cultivated year-round in Benin. This crop is grown either in monoculture or in mixed culture with other vegetables and maize. FAW larvae have been frequently found associated with onion during both maize growing and off seasons (Winsou et al. 2022). According to Bale et al. (2002), an important aspect of successful herbivore adaptation to a host plant and climatic environment is determined by its ability to complete its life cycle.

Determining reproduction of a pest species in relation to the host plants is crucial to understanding pest population dynamics throughout the year. It also reveals the pest’s survival strategy to adapt to the absence of the preferred host plant by exploiting alternative hosts in newly invaded environments. Several studies are available reporting the effects of temperature on FAW development and reproduction over a wide temperature range, but to the best of our knowledge these studies are often limited to maize (Du Plessis et al., 2020; Prasad et al., 2021; Simmons, 1993) and very few alternative crops (Barros et al., 2010; He et al., 2021).

The polyphagous nature of FAW and its capacity to maintain its population on alternative host plants during maize off season is crucial information required for managing the pest successfully (Winsou et al. 2022). In this study, we investigated the reproduction potential of FAW on onion, which could shed new light on the pest's capability to perpetuate its population in the absence of maize plants.

Materials and Methods

Insect colonies

FAW egg masses and larvae were collected from maize and onion fields in southern Benin. The samples were contained in plastic boxes and taken to the laboratory for screening. Pure colonies of FAW were established on the host plants of their origin, either maize or onion, in the laboratory of the Biorisk Management Facility (BIMAF) at the International Institute of Tropical Agriculture (IITA), Benin station.

Rearing insects on natural diet for the experiments

Natural diets were used for FAW rearing according to a mass production protocol established earlier at BIMAF. In brief, FAW larvae were fed with either fresh sprouting maize (variety EVDT) or onion leaves (variety SAFARI) and reared to the pupal and adult stages. The food was surface sterilized with 5% antimicrobial sodium hypochlorite solution and rinsed several times with abundant distilled water to wash-off the bleach before feeding FAW larvae. The pupae were collected daily and disinfected in a similar manner and placed in oviposition boxes (18 cm diameter, 10 cm height). The oviposition boxes were covered with cotton mesh until the pupae hatched to adult. The emerged moths were allowed to mate in the oviposition boxes and fed with a 10% honey solution dispensed in capsules of cotton wool. After mating, FAW females laid their eggs (in masses) on a cover of cotton mesh and on the oviposition box wall approximately two days post-emergence. FAW eggs and egg masses were collected every two days and incubated in new rearing boxes containing sterile sprouting maize or onion leaves. The rearing conditions were 26 ± 1 °C temperature, 65 ± 5% relative humidity and 12 hours photophase. The eggs hatched within two to three days and the neonate caterpillars started feeding. The larval stage (with six instars) lasted about fifteen days. The approximate pupal stage duration was about seven days.

Development of immature stages and survival

All experiments were conducted in external shelters at ambient fluctuating temperatures. Temperature data loggers (Elitech RC 5, China) were used to record the temperature variation in the shelter. Newly laid eggs were placed individually in small Petri dishes (5.5cm diameter, 1.3cm height), sealed with parafilm and incubated in the shelter at ambient fluctuating temperatures. One hundred (100) individual eggs were followed on each host plant (maize or onion) at ambient temperature until adult emergence. The observations were done once a day to check and record the mortality or moult to subsequent stage. Unhatched eggs were followed for a maximum period of thirty days before concluding on incapacity to develop. Newly emerged larvae were individually transferred into small rearing boxes with a perforated lid covered with muslin (4.2 cm diameter; 5.3 cm height) and fed with either sprouting maize or onion leaf depending on the experimental host crop. Larval survival was recorded daily until pupation. Newly formed pupae were transferred to another rearing box of the same size and incubated. Emerging adults were counted and sexed at daily observations.

Adult longevity and reproduction

Freshly emerged adults collected from the mass rearing were used in the experiments. The moths were conditioned in small rearing boxes (diameter 12 cm, height 8 cm) and sustained with a solution of 10% honey. A total of 30 couples from rearing cohorts previously fed with either sprouting maize or onion leaf at their larval stages (depending on which plant they were collected from) were tested at the ambient fluctuating temperatures. After mating, the number of eggs and egg masses laid by females were counted daily. Both sexes were followed until they died.

Data analysis

The life table parameters of *Spodoptera frugiperda* (Table 3) were calculated at ambient temperatures with the aid of statistical analysis tool SAS (SAS 2013) and using the program developed by (Maia et al. 2000). The two cohorts of experimental insects collected either from maize or onion fields and fed at the larval stage with host plant of origin were analysed separately.

Results

Stage specific development time and survivorship

Spodoptera frugiperda successfully completed its life cycle on its prime host (maize) and on its most common alternative host plant (onion). The egg-adult development time on maize was 25.76 ± 0.22 days, 9 days earlier compared to onion (Table 1). Almost all eggs laid hatched on both host plants. However, there was a high larval mortality on onion, this resulted in only 12.24% of individuals reaching adulthood compared to maize (63.44%).

Table 1. Stage specific development time and survival rate of FAW on maize and onion at ambient temperatures.

Parameter		**Host plant**	
		Maize	**Onion**
Development time	Egg	2.00 ± 0.00	2.03 ± 0.01
	Larva	12.50 ± 0.40	10.63 ± 0.98
	Pupa	8.42 ± 0.25	8.78 ± 0.61
	Egg-adult	25.76 ± 0.22	36.25 ± 0.37
Survival rate (%)	Egg	93.00	98.00
	Larva	71.42	16.32
	Pupa	95.21	73.52
	Adult	61.96	100
	Egg-adult	63.44	12.24

Reproduction of FAW on maize and onion

Pre-oviposition, oviposition and post-oviposition periods were almost equal for females reared on maize and onion during their larval stage (Table 2). The same trends were observed for both female and male longevity. Females reared on maize were the most prolific, with 1267.46 eggs compared to 854.36 on onion. The proportion of female offspring of parents reared on maize was relatively higher with 0.61 vs. 0.5 on onion.

Table 2. Reproduction parameters (mean ± SE) of FAW on maize and onion at ambient temperatures.

Parameter	Host plant	
	Maize	**Onion**
Pre-oviposition period (days)	2.83 ± 0.42	2.53 ± 0.31
Oviposition period (days)	6.06 ± 0.34	6.15 ± 0.71
Post-oviposition period (days)	1.76 ± 0.26	2.07 ± 0.42
Female longevity (days)	10.60 ± 0.46	10.62 ± 0.67
Male longevity (days)	9.83 ± 0.71	10.63 ± 0.85
Lifetime fecundity (offspring/ female)	1267.46 ± 88.16	854.36 ± 102.75
Sex ratio (proportion of females progeny)	0.61	0.50

Population growth parameters of FAW on maize and onion

The net reproductive rate (R_o), the intrinsic (r_m) and finite (λ) rates of increase were relatively higher on maize (R_o 499.92; r_m 0.27; λ 1.32) compared to onion (R_o 63.77; r_m 0.13; λ 1.14), (Table 3). The development of FAW fed on onion at the larval stage was delayed at ambient temperatures (Table 3 and Figure 1). Females on both maize and onion displayed a unimodal fertility pattern with the highest number of eggs laid by females reared on maize during their larval stage. The maximum eggs laid by females previously reared on maize occurred on day 26, one week earlier compared to females previously fed on onion during their larval stage.

Table 3. Life table parameters of *Spodoptera frugiperda* on two host plants (maize and onion) at ambient fluctuating temperatures.

Parameter	Host plant for *Spodoptera frugiperda* **(mean [95% confidence interval])**	
	Maize	**Onion**
Net reproductive rate, Ro (progeny per female)	499.92 [373.83, 626.02]	63.77 [37.99, 89.56]
Intrinsic rate of increase, r_m (per day)	0.27 [0.26, 0.29]	0.13 [0.12, 0.14]
Doubling time, Dt (days)	2.48 [2.34, 2.61]	5.15 [4.66, 5.63]
Mean generation time, T (days)	22.26 [21.50, 23.03]	30.98 [29.31, 32.66]
Finite rate of increase, λ	1.32 [1.30, 1.34]	1.14 [1.12, 1.15]

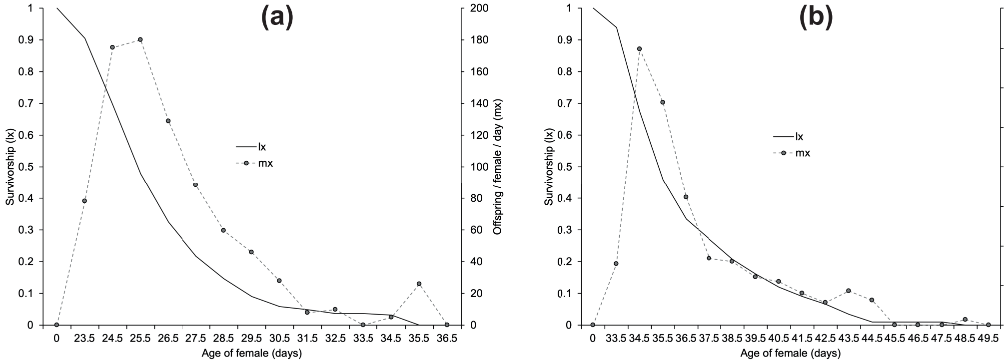

Figure 1. Fertility (mx) and age-specific survival (lx) of *Spodoptera frugiperda* fed with (a) maize or (b) onion during the larval stage at ambient fluctuating temperatures.

Discussion

Temperature is one of the most fundamental bioclimatic variables to describe poikilothermic organisms' development. The knowledge of thermal requirements for insect development is central to predict distribution and migration patterns (Tepa-Yotto et al. 2021, Guimapi et al. 2022). The present work addresses existing gaps on FAW's capability to survive on alternative host plants at varying ambient temperatures.

Spodoptera frugiperda was able to complete its life cycle on onion as one of its alternative host plants in southern and central Benin, demonstrating that onion is a suitable host plant for FAW. Insect herbivore adaptation to an alternative host plant allows the pest to maintain its offspring until the preferred plant is available (Bale et al. 2002). Other possible mechanisms of survival in the absence of the preferred host plant include the ability of FAW to complete its life cycle by crawling or ballooning using secreted silk whereby causing herbivory from crop to crop in nature (Casmuz et al., 2010; Winsou et al., 2022). Despite the poor survival rate of FAW on onion, these laboratory results indicate that the pest can use onion as an alternative food substrate between maize growing seasons, as earlier observed by Winsou et al., (2022) and Cokola et al., (2021). Nevertheless, the lower numbers of offspring obtained from onion might support earlier statements that onion has antinutritional properties and can be included in an IPM package against the pest (Munyore and Rioba 2020).

During earlier sampling efforts on the field, FAW larvae were often found associated with onion, particularly in vegetable production systems (Winsou et al. 2022). Therefore, the relative unsuitability of onion for the insect in this study might be two-fold. It is not new that an insect's fitness can differ across host plant species and over varieties of the same host plant species (He et al., 2021). Furthermore, there is some literature to support that striving in unsuitable flora, females can trade-off and lay eggs to increase the likelihood of offspring survival and perennity on less suitable host plants (Cokola et al. 2021). Indeed, intensified efforts were required to obtain large numbers of individuals of FAW reared on onion for our experiments. In Benin, like in several other African countries, the maize strain of FAW is the most abundant (99%) with some hybrids and very few of the rice strain (Nagoshi et al. 2022). How this can affect adaptation and survival of the pest on alternative host plants deserves more scrutiny. In addition, the behavioural strategy of older FAW larvae to hide in the maize whorl and the lack of of sheltering organs in the morphology of some other host plants to enable successful pest development completion is another gap to document. During previous field sampling, the window pane like symptom was the most common that indicated the presence of FAW larvae inside on onion leaf (Winsou et al. 2022). FAW larvae can hide in the tubal onion leaves but may only hide under leaves of other alternative plants that lack sheltering organs. The survival or ability of FAW to complete its development in alternative plants while exposed to disturbances including abiotic factors and higher trophic level organisms needs to be assessed. Another explanation to the unsuitability of onion for FAW in this study might be linked to some insecticidal properties of some of the contents of onion leaves. In effect, methanolic extracts of onion proved toxic and as effective as synthetic chlorantraniliprole against FAW larvae in earlier reports (Munyore & Rioba, 2020).

In conclusion, the study provides baseline data for further FAW phenology model validation and risk mapping on both maize and onion. FAW fed on onion during the larval stage could complete its development and reproduce, indicating the potential of this host plant to sustain populations of FAW during the off-season. Onion as a potential host plant of FAW may also constitute a reservoir of natural enemies which might influence the success of augmentative releases and conservation biological control. Therefore, the insights could be exploited in the management of FAW through the use of onion intercropped with maize. However, there is no silver bullet solution in the management of FAW, which suggests the use of integrated approaches including biopesticides and biological control.

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Paper III

Phenology and functional response of the egg parasitoid *Telenomus remus*: comparison of local and exotic parasitoid strains at three constant temperatures for biocontrol decision support

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Abstract

The fall armyworm (FAW) *Spodoptera frugiperda* (Lepidoptera: Noctuidae) has become a priority pest in Benin as in other countries in Africa. This work was conducted to give a basis for decision support for introductions or augmentation/conservation of *Telenomus remus* Nixon (Hymenoptera: Scelionidae) in Benin. We hypothesized that i) temperature equally affects the biological characteristics of the two strains (local and exotic) of *T. remus*; ii) the life table statistics of the two strains of *T. remus* are not significantly different at varying prey densities under three constant experimental temperatures; and iii) the efficacy of the two strains of *T. remus* is parasitoid density-dependent at ambient temperatures. The local female parasitoid strain was relatively the most prolific with on average 159.0 FAW eggs parasitized per female at 31 °C. Lifetime fecundity was equal between the two parasitoid strains at 25 °C, 28 °C and 31 °C and both local or exotic parasitoid strains sex ratio were not significantly influenced by the temperature. Most of the life table parameters of *T. remus* were statistically equal between local and exotic strains at the various constant experimental temperatures. Both parasitoid strains displayed relatively higher intrinsic rate of increase (r_m per day) at 31 °C, local $r_m = 0.37$ and exotic $r_m = 0.44$. The number of parasitized eggs increased while the percentage of eggs parasitized was inversely proportional to the FAW egg density, displaying type II functional response patterns. In general, female parasitoid densities beyond 5 wasps did not significantly improve parasitoid performance on clusters of 100-200 FAW eggs. Although it may be considered by some to be unnecessary to import and release *T. remus*, we conclude that it could be advantageous to opportunistically recruit exotic breeds to complement the action of local counterparts during colder periods.

Keywords: *Telenomus remus*, parasitoid strain, biological parameters, life tables, parasitoid performance.

Introduction

The fall armyworm (FAW) *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae) is a highly polyphagous and voracious pest originating from the Americas (Cave, 2000). Fall armyworm was first reported in 2016 in West Africa and spread within few years over the entire continent with severe consequences on maize production systems including in Benin (FAO, 2021; Goergen et al., 2016; Houngbo et al., 2020; Koffi et al., 2020; Montezano et al., 2018; Tindo et al., 2017). Thus, the initial outbreaks of FAW in Africa were and continue to be associated with huge socio-economic costs. The species rapidly became one of the costliest agricultural threats on the continent and caused losses estimated to be nearly 10 billion US dollars per annum (Eschen et al., 2021). In Benin, respondents to a field survey conducted in 2018 concurred that FAW is responsible for yield losses of 797.2 kg/ha in maize fields, representing 49% of the average maize yield commonly obtained by farmers (Houngbo et al., 2020). As a result, the food security and livelihoods of millions of farmers are put at risk. Contingency plans including farmers' solutions initially relied on the use of insecticides, some of which are highly hazardous. Subsequent research for development strategies explored much sounder and evidence-based integrated pest management strategies including classical biological control (Sisay et al., 2018). The classical biological control approaches initially envisioned expeditions to Latin America with plans to import and release specific promising parasitoid species including the egg parasitoid *Telenomus remus* Nixon (Hymenoptera: Scelionidae). However, the discovery of local strains of *T. remus* led to the reconsideration of classical biological control plans for Africa (Tepa-Yotto et al., 2022).

Telenomus remus is referenced in multiple literature sources to be a prolific and efficient egg parasitoid of lepidopterous insects. The parasitoid originated from Papua New Guinea and the peninsular Malaysia where it was described in Ulu Gombak, just NE of Kuala Lumpur (Wengrat et al., 2021). This parasitoid is an important and efficient biological control agent against many pests, particularly those of the genus *Spodoptera* (Noctuidae) including *S. frugiperda* in the Americas and elsewhere (Cave, 2000; Colmenarez et al., 2022). Some previous sampling efforts in Benin reported the multi-species egg parasitoid *Telenomus* spp. association with the maize stemborer *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) (Schulthess et al., 2001) but no specific mention was made about *T. remus.* Recent studies report a number of local parasitoid species that have adapted to FAW in Benin (Agboyi et al., 2020; Kenis et al., 2019; Winsou et al., 2022) and elsewhere, among which the most promising egg parasitoid is *T. remus*. This egg parasitoid was frequently found on FAW egg collected from maize which is a prime host plant of FAW and on many other alternative host plants as predicted by earlier modelling efforts (Tepa-Yotto et al., 2021).

In West Africa (Ghana and Niger), some field release experiments have been conducted using the locally recruited *T. remus* (Agboyi et al., 2021; Laminou et al., 2020). However, the development of an efficient biological control program promoting parasitoids must involve screening a range of candidate species followed by selection of high efficiency strains with minor non-target effects. Classical biological control would only be considered for implementation in instances where the local biodiversity doesn't offer competitive candidate parasitoid species. It was, therefore, crucial to compare the efficiency level of a candidate *T. remus* strain from the Americas with that of a Benin local strain by studying their life table parameters, biological characteristics and functional responses. The work was conducted to give decision support for considering introductions or augmentation/conservation of *T. remus* in Benin. We hypothesized that i) temperature equally affects the biological characteristics of the two strains (local and exotic) of *T. remus*; ii) the life table statistics of the two strains of the parasitoid are not significantly different at varying prey density under three constant experimental temperatures; and iii) the efficacy of the two strains of *T. remus* is parasitoid density-dependent at ambient temperatures.

Materials and Methods

Insect colonies

The fall armyworm colonies used in this experiment originated from collections on maize fields in southern Benin. They were mass-reared following the protocol developed in earlier work (Winsou et al, unpublished data) under laboratory conditions, 26 ± 1°C, 60 ± 5% relative humidity and a photophase of 12 hours light and 12 hours dark. Briefly, FAW caterpillars were fed with fresh, sterilized sprouting maize (variety EVDT) and reared to pupal stage and adulthood. The emerging moths were fed with a 10% honey solution imbibed from cotton wool.

The egg parasitoid *T. remus* was collected locally on maize fields in southern Benin. The initial cohort was maintained on FAW egg masses. The parasitoid was subsequently offered FAW eggs *ad libitum* and mass reared to produce enough offspring for the experiments. The exotic strain of *T. remus* was imported from USDA-ARS CMAVE, Insect Behavior and Biocontrol Research Unit, Gainesville, Florida 32608, USA. They were kept in strict isolation facilities using local *S. frugiperda* eggs as the host. The exotic parasitoids were also massively host and mass reared under the same laboratory conditions as for the local strain. In both cases adult parasitoids were fed with honey droplets. To ensure that the two strains were kept strictly separated, the experiments were conducted in different rooms and separate growing chambers. The offspring of the emerging parasitoids were also kept in the growing chamber until they died.

Experiment 1. Parasitoid life tables at constant temperatures

Insect incubators were set to three separate constant experimental temperatures (25, 28 and 31°C), 65 ± 5% relative humidity and a 12-hour phase light/dark cycles. Temperature data loggers (Elitech RC 5, China) were used in the incubators to monitor temperature accuracy.

All data used in the analysis were quality checked in terms of matching to the exact experimental temperature. Fall armyworm egg cards were prepared for the experiments by gluing the eggs with an odorless and non-toxic transparent glue. They were then exposed to less than 12-hour old mated females of *T. remus* (Carneiro et al., 2010) in glass vials (8.5 cm height and 3.0 cm diameter). One egg card of FAW was exposed to one female parasitoid per each glass vial. The vials were covered with tight micromesh tissue to avoid the parasitoid from escaping and they were closed using a perforated lid for ventilation purposes. Experimental female parasitoids were fed with honey droplets. Female parasitoids of the local strain were compared to the exotic strain and their performance was measured individually on a 100 FAW eggs cluster glued on a cardboard (6 cm length and 2.5 cm width). The egg card was removed from the vials after 24 hours exposure to the parasitoid and replaced by a new egg card until the female parasitoid died. The parasitized eggs were checked for successful parasitism under a stereomicroscope (Chen et al., 2021) and kept at the same temperature until adult emergence for daily observations and measurements of development parameters and lifespan. There were 10 replicates of the female parasitoids for each strain and temperature combination, giving a total 60 wasps used. Females that died before 24 hours post-exposure were discarded from the data analysis.

Experiment 2. Parasitoid functional response at constant temperatures

One individual mated female (< 12 hours age) of each of the two strains of *T. remus* was confined with fresh eggs of *Spodoptera frugiperda* (< 24 hours old) at various egg densities (10; 30; 50; 70; 100; 150 and 200) on cardboards as described by Pomari et al. (2012) at three distinct constant temperatures. All of the experiments were conducted in insect growth incubators (FITOCLIMA 1200 PLH, Aralab Rio de Muro, Portugal) at 12L:12D photophase and 65-75% relative humidity. Parasitism was measured after 24 hours of exposure of the FAW egg clusters to female wasps. Observations were done by dissection of egg masses under a stereomicroscope (Chen et al., 2021) to confirm successful parasitism. There were 10 replicates of the female parasitoids at each experimental host density for the respective temperatures, giving a total of 210 wasps tested. However, the wasps that died before the 24 hours experiment duration were discarded from the analysis.

Experiment 3. Telenomus remus performance at various parasitoid densities

Five densities (1, 5, 10, 15, 20) of 12-hour old mated females of *T. remus* were tested on masses of 100-200 eggs of *S. frugiperda* at ambient temperatures fluctuating from 22 to 30 °C, on average 25.5 ± 0.03 °C. Parasitoids at each density were confined with an egg mass in glass vials for 24 hours. The number of eggs in an egg mass was estimated by multiplying the number of eggs on the outer layer by the number of inner layers of each egg mass. The number of eggs on the edges was added to the total (Beserra & Parra, 2005). There were 10 combinations of two parasitoid strains (local and exotic) and five parasitoid densities (1, 5, 10, 15, 20 mated females) (Pomari et al., 2013). The number of replicates was 10, giving a total number of 100 female parasitoids. Total parasitism and resulting adult emergence were calculated for each parasitoid density.

Data collection and analysis

For each experiment, the eggs exposed to *T. remus* were followed for the first four days to remove the hatching FAW larvae and throughout the experiment to count the parasitized eggs. The offspring male and female parasitoids were counted upon emergence to calculate the sex ratio.

The number of FAW eggs parasitized by *T. remus* (Tables 1 & 5) were $\log_{10}(y + 1)$ transformed where necessary before analysis to meet the assumptions of normality and equal variance. Series of one-way analysis of variance (ANOVAs) followed by Tukey's post hoc tests at the 5% level and by pairwise comparisons were performed to identify significant differences (R Core Team, 2012).

The life table parameters of *T. remus* (Tables 2-4) were calculated at three constant temperatures using the SAS program developed by Maia et al. (2000). Differences in the intrinsic rate of increase values were tested for significance by estimating variances through the jackknife method (Meyer et al., 1986). The life table parameters were compared between local and exotic strains of *T. remus* by performing ANOVA using the GLM procedure in SAS followed by paired Student's t-tests (SAS, 2013)

For the functional response study, binary logistic regression with a logit link function was used to test for the effect of parasitoid strain as a factor and that of *S. frugiperda*'s egg mass density as a continuous explanatory variable on *T. remus* parasitism at different constant temperatures (Minitab, 2011) . The Fisher's exact tests with Bonferroni correction at the 5% level were also performed to test for significant differences among the parasitoid strains (local and exotic), followed by pairwise comparisons.

Results

Parasitoid life tables at constant temperatures

Telenomus remus biological parameters were diversely affected by temperature and parasitoid strain factors (Table 1).

The local parasitoid strain had longer egg-adult development time (13.0 days) at 25 °C ($F_{1,212} = 25.9$; $P < 0.0001$), shorter at 28 °C ($F_{1,135} = 15.2$; $P = 0.0001$) and equal at 31 °C ($F_{1,160} = 3.01$; $P = 0.084$) when compared to the exotic parasitoid strain (Table 1). The higher the temperature, the shorter the egg-adult time for both the local ($F_{2,253} = 1317.6$; $P < 0.0001$) and the exotic ($F_{2,254} = 743.4$; $P < 0.0001$) parasitoid strains. The fastest egg-adult development time was 9.0 days for the exotic parasitoid strain at 31 °C.

Overall, the oviposition time on average ranged from 5.2 (local parasitoid strain at 28 °C) to 10.8 (local parasitoid strain at 25 °C) days (Table 1). There were no statistically significant differences in oviposition time between the local and the exotic parasitoid strains at any of the temperatures tested (($F_{1,26} = 0.80$; $P = 0.378$), ($F_{1,25} = 3.19$; $P = 0.085$), ($F_{1,25} = 3.85$; $P = 0.060$) for 25; 28; and 31 °C, respectively). The lower the temperature, the longer the oviposition time for the local parasitoid strain ($F_{2,38} = 6.86$; $P = 0.002$); but that was not the case for the exotic parasitoid strain which had stable oviposition time independent of the temperature tested ($F_{2,38} = 1.66$; $P = 0.202$).

The mean post-oviposition time was generally 1.0-2.7 days (Table 1). This did not change significantly between the two parasitoid strains (local and exotic) at any of the constant temperatures (($F_{1,28} = 0.15$; $P = 0.696$), ($F_{1,25} = 2.02$; $P = 0.166$), ($F_{1,25} = 0.59$; $P = 0.449$) for 25; 28; and 31 °C, respectively); nor between temperatures for a given parasitoid strain, local ($F_{2,38} = 2.10$; $P = 0.135$) or exotic ($F_{2,40} = 1.63$; $P = 0.207$).

Females of both *T. remus* strains (local and exotic) generally lived for a shorter time with increasing temperature, but the same parameter (7.84-13.13 days) showed no significant differences between the two strains at any of the constant temperatures ($P > 0.05$) (Table 1).

The local female parasitoid strain was relatively the most prolific at 31 °C with on average 159.0 FAW eggs parasitized per female parasitoid. Female lifetime fecundity was equal between the two parasitoid strains at 25 °C ($F_{1,28} = 1.18$; $P = 0.286$), 28 °C ($F_{1,25} = 0.02$; $P = 0.884$) and 31 °C ($F_{1,25} = 0.15$; $P = 0.694$). Temperature did not affect the female exotic parasitoid strain's lifetime fecundity ($F_{2,40} = 0.05$; $P = 0.949$), nor for the local parasitoid strain, with the highest number of FAW eggs parasitized per female parasitoid at 31 °C ($F_{2,38} = 1.07$; $P = 0.355$).

Parasitoid viability (93.6-98.3 %) was not different between the two strains at any of the tested temperatures, ($F_{1,28} = 0.70$; $P = 0.406$), ($F_{1,25} = 3.76$; $P = 0.063$), ($F_{1,25} = 0.96$; $P = 0.334$) at 25; 28; and 31 °C, respectively; nor was the effect of temperature on adult emergence rate of both parasitoid strains, local ($F_{2,38} = 0.40$; $P = 0.668$) and exotic ($F_{2,40} = 0.64$; $P = 0.531$).

The highest female proportion in the progeny of *T. remus* (0.74) was observed on the exotic parasitoid strain. This was greater than that of the local strain at all temperatures tested (($F_{1,28} = 15.70$; $P = 0.0004$), ($F_{1,25} = 8.84$; $P = 0.006$), ($F_{1,25} = 24.54$; $P < 0.0001$) at 25; 28; and 31 °C, respectively). However, temperature did not significantly affect the sex ratio of the local ($F_{2,38} = 1.92$; $P = 0.159$) or exotic ($F_{2,40} = 1.40$; $P = 0.257$) parasitoid strains.

Most of the life table parameters of *T. remus* did not show statistically significant differences between local and exotic strains at the various constant experimental temperatures (Tables 2-4; Figure 1). The relatively higher net reproductive rates (*Ro*) were recorded for the local parasitoid strain (120.8) and for the exotic specimens (120.6 progeny per mother), at 31 and 28 °C, respectively, and without any significant differences between the two strains at any temperature tested. The remaining life table parameters showed statistically significant differences between the two parasitoid strains at 25 °C only, with the exotic females generally being the most prolific relatively speaking (Tables 2-4). Both parasitoid strains displayed relatively higher intrinsic rate of increase (r_m per day) at 31 °C, local $r_m = 0.37$ and exotic $r_m = 0.44$.

Table 1. Development time (mean ± SE), oviposition time (mean ± SE), longevity (mean ± SE), female lifetime fecundity (mean ± SE), adult emergence rate (mean ± SE) and sex ratio (mean ± SE) of two strains of *Telenomus remus* at three different temperatures (at host density 100).

Parameters	**Temperature regimes**					
	25 °C		**28 °C**		**31 °C**	
	Local	**Exotic**	**Local**	**Exotic**	**Local**	**Exotic**
Egg-adult time (days)	13.009 ± 0.06 Bc	12.52 ± 0.06 Ac	10.80 ± 0.07 Ab	11.22 ± 0.06 Bb	9.08 ± 0.03 Aa	9.0 ± 0.0 Aa
Oviposition time (days)	10.80 ± 1.27 Ab	9.30 ± 1.01 Aa	5.25 ± 1.05 Aa	8.33 ± 1.28 Aa	8.57 ± 0.67 Aab	6.64 ± 0.84 Aa
Post-oviposition time (days)	2.33 ± 0.75 Aa	2.73 ± 0.67 Aa	1.0 ± 0.21 Aa	1.86 ± 0.51 Aa	1.07 ± 0.32 Aa	1.38 ± 0.24 Aa
Female longevity	13.13 ± 1.64 Aa	12.33 ± 1.20 Aa	10.2 ± 0.96 Aa	10.2 ± 1.25 Aab	9.64 ± 0.70 Aab	7.84 ± 0.79 Ab
Lifetime fecundity (egg parasitized/ female)	116.86 ± 12.19 Aa	147.33 ± 14.07 Aa	140.2 ± 43.02 Aa	146.53 ± 20.30 Aa	159.0 ± 25.51 Aa	140.92 ± 15.34 Aa
Adult emergence rate (%) (percentage of emergence (viability))	96.01 ± 2.25 Aa	93.63 ± 1.71 Aa	98.33 ± 0.96 Aa	95.58 ± 1.04 Aa	97.65 ± 1.88 Aa	95.40 ± 1.23 Aa
Sex ratio (rate females' progeny)	0.57 ± 0.02 Ba	0.71 ± 0.02 Aa	0.41 ± 0.08 Ba	0.67 ± 0.03 Aa	0.50 ± 0.04 Ba	0.74 ± 0.01 Aa

Means of local and exotic parasitoid strains at a given temperature followed by different uppercase letters are significantly different (Tukey's tests at the 5% level). Means of a given parasitoid strain at various temperatures followed by different lowercase letters are significantly different (Tukey's tests at the 5% level).

Table 2. Life table parameters of locally recruited and exotic strains of *Telenomus remus* (local and exotic) at 25 °C.

Parameters	***Telenomus remus* (mean [95% confidence interval])**		***P****
	Local	**Exotic**	
Net reproductive rate, Ro (progeny per female)	74.51 [60.90, 88.13]	104.27 [82.03, 126.51]	0.988
Intrinsic rate of increase, r_m (per day)	0.34 [0.33, 0.36]	0.41 [0.39, 0.44]	0.0001
Doubling time, Dt (days)	1.98 [1.88, 2.08]	1.65 [1.57, 1.73]	0.0001
Mean generation time, T (days)	12.35 [11.75, 12.94]	11.08 [10.78, 11.39]	0.0003
Finite rate of increase, λ	1.41 [1.39, 1.44]	1.52 [1.48, 1.55]	0.0001

* *P*-values indicating statistical differences between local and exotic *Telenomus remus* strains, according to the paired Student's *t*-tests at the 5 % level.

Table 3. Life table parameters of the two strains of *Telenomus remus* (local and exotic) at 28 °C

Parameters	***Telenomus remus* (mean [95% confidence interval])**		***P****
	Local	**Exotic**	
Net reproductive rate, Ro (female per female)	52.41 [15.78, 89.04]	120.61 [78.04, 163.17]	0.992
Intrinsic rate of increase, r_m (per day)	0.36 [0.29, 0.42]	0.37 [0.29, 0.45]	0.616
Doubling time, Dt (days)	1.90 [1.55, 2.25]	1.82 [1.45, 2.19]	0.375
Mean generation time, T (days)	11.08 [10.18, 11.98]	12.63 [9.43, 15.82]	0.833
Finite rate of increase, λ	1.43 [1.34, 1.52]	1.45 [1.33, 1.57]	0.613

* *P*-values indicating statistical differences between local and exotic *Telenomus remus* strains, according to the paired Student's *t*-tests at the 5 % level.

Table 4. Life table parameters of the two strains of *Telenomus remus* (local and exotic) at 31 °C

Parameters	***Telenomus remus* (mean [95% confidence interval])**		***P****
	Local	**Exotic**	
Net reproductive rate, Ro (female per female)	120.80 [52.63, 188.97]	83.20 [66.40, 100.01]	0.133
Intrinsic rate of increase, r_m (per day)	0.37 [0.27, 0.47]	0.44 [0.41, 0.48]	0.931
Doubling time, Dt (days)	1.82 [1.45, 2.18]	1.54 [1.42, 1.66]	0.067
Mean generation time, T (days)	12.66 [8.69, 16.64]	9.85 [9.24, 10.45]	0.077
Finite rate of increase, λ	1.44 [1.29, 1.60]	1.56 [1.51, 1.62]	0.930

* *P*-values indicating statistical differences between local and exotic *Telenomus remus* strains, according to the paired Student's *t*-tests at the 5 % level.

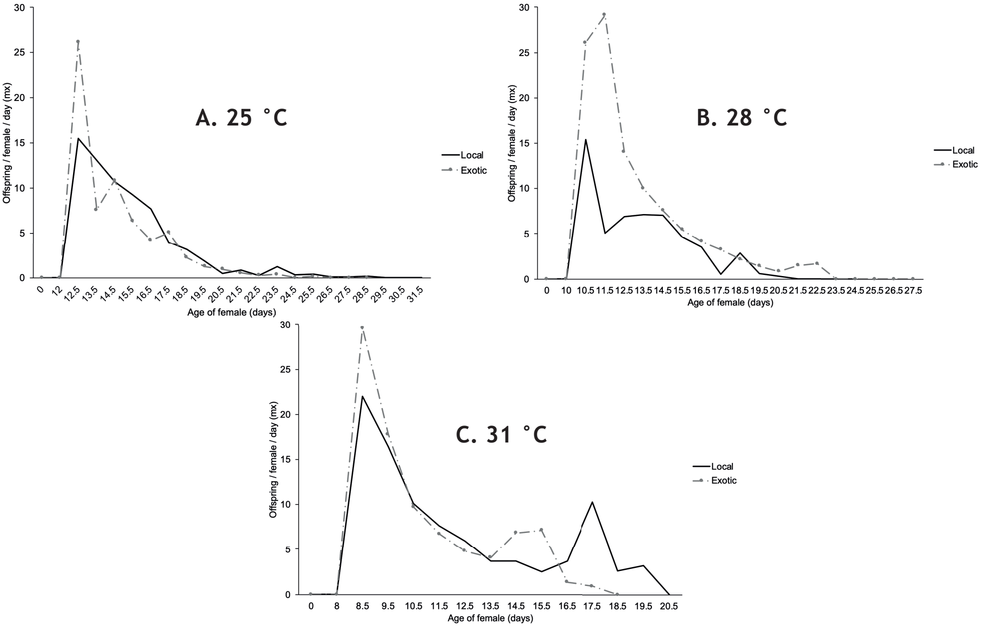

Figure 1. Fecundity of local and exotic strain of *T. remus* at (A) 25, (B) 28 and (C) 31 °C.

Parasitoid functional response at constant temperatures

The binary logistic regression provided a good prediction of *T. remus* strain effect and FAW egg density on the parasitoid oviposition at three different constant temperatures (Tables 5 and 6). The number of eggs parasitized increased while the percentage of eggs parasitized was inversely proportional to the FAW egg density, displaying type II functional response patterns (Figures 2-4). The oviposition of the exotic strain of *T. remus* was higher than that of the local strain at both 25 and 28 °C, while the local parasitoid strain was superior in warmer conditions (31 °C) (Figures 2-4). In all instances, there was evidence that both the parasitoid strain as a factor and the host egg density as a continuous explanatory variable were significantly different from zero for the response investigated (Tables 5 and 6), showing that the two variables affected *T. remus* oviposition. However, there was no interaction between these two effects.

Table 5. Binary logistic regression of two *Telenomus remus* strains (local and exotic) and FAW egg density on parasitism[1]

Predictor	Coefficient ± SE	*Z*	Odds ratio (95% confidence interval)[2]
Parasitized eggs at 25 °C			
Constant	0.124651 ± 0.0339294***	3.67	
Parasitoid strain (slope)	0.782294 ± 0.0292192***	26.77	2.19 (2.06 - 2.32)
FAW egg density (slope)	-0.0054041 ± 0.0002586***	-20.90	0.99 (0.99 - 1.00)
Parasitized eggs at 28 °C			
Constant	0.884223 ± 0.0412113***	21.46	
Parasitoid strain (slope)	0.539318 ± 0.0348454***	15.48	1.71 (1.60 - 1.84)
FAW egg density (slope)	-0.0085353 ± 0.0002710***	-31.49	0.99 (0.99 - 0.99)
Parasitized eggs at 31 °C			
Constant	0.661302 ± 0.0343263***	19.27	
Parasitoid strain (slope)	-0.129224 ± 0.0279972***	-4.62	0.88 (0.83 - 0.93)
FAW egg density (slope)	-0.0044197 ± 0.0002418***	-18.28	1.00 (1.00 - 1.00)

[1] Binary logistic regression models with replicate included as an additional factor did not lead to qualitatively different results, therefore the data were pooled. [2] Odds ratio means the estimated probability for parasitism to be successful/unsuccessful for local compared to exotic strain of *T. remus*, and for each increase of host FAW egg density.

*** Denotes $p < 0.0001$.

Table 6. Results of goodness-of-fit tests related to the results of binary logistic regressions in Table 5.

Experiment	Goodness-of-fit test	χ^2	df
Parasitized eggs at 25 °C			
	Pearson	535.965***	11
	Deviance	551.911***	11
Parasitized eggs at 28 °C			
	Pearson	560.542***	11
	Deviance	615.339***	11
Parasitized eggs at 31 °C			
	Pearson	466.355***	11
	Deviance	523.404***	11

*** Denotes $p < 0.0001$.

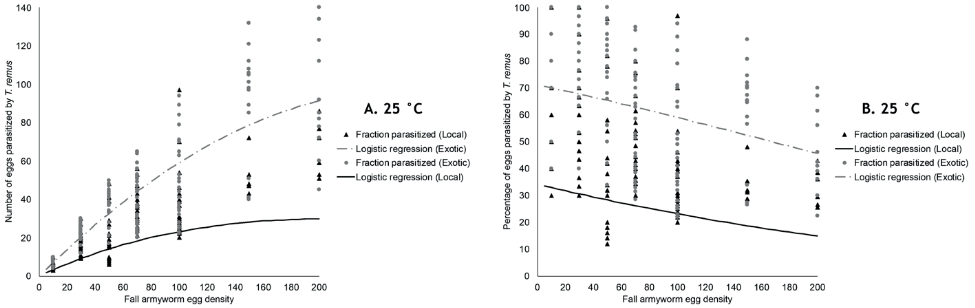

Figure 2. Number (A) and percentage (B) of fall armyworm eggs parasitized by the local and the exotic strains of *T. remus* at 25 °C.

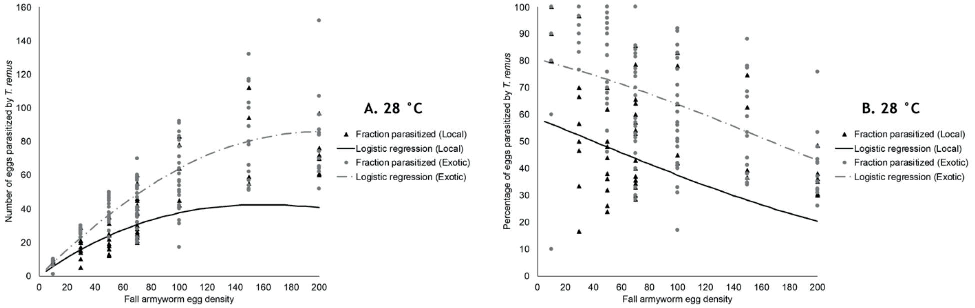

Figure 3. Number (A) and percentage (B) of fall armyworm eggs parasitized by the local and the exotic strains of *T. remus* at 28 °C.

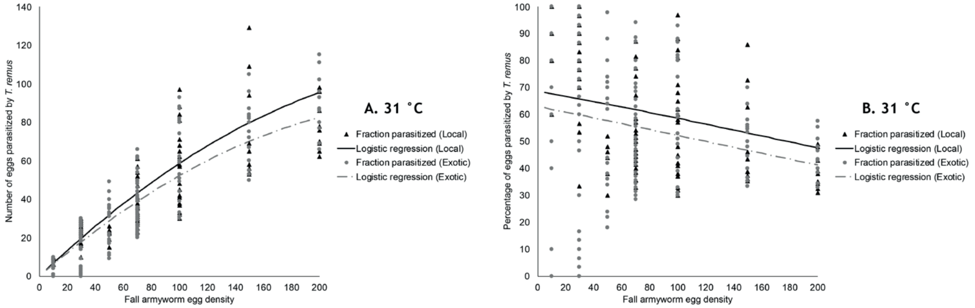

Figure 4. Number (A) and percentage (B) of fall armyworm eggs parasitized by the local and the exotic strains of *T. remus* at 31 °C.

Telenomus remus performance at various parasitoid densities

The performance of *T. remus* females (either local or exotic strain) on 100-200 egg masses exposed to different parasitoid densities (1; 5; 10; 15 and 20 wasps), varied at ambient temperature ($P < 0.05$; Table 5). Differences were only occasionally observed between the two strains at the respective parasitoid densities.

The total parasitism rate was relatively higher (107.2 FAW eggs parasitized) for the local parasitoid strain at a density of 5 female wasps compared with 101.35 eggs parasitized for the exotic strain (Table 7), but there were no statistically significant differences across the different parasitoid densities ($F_{4,70} = 2.13$; $P = 0.086$). Conversely, the parasitism rate was significantly lower (39.4 FAW eggs parasitized) at the 1 female wasp density of the exotic strain, 2.7-fold smaller than the highest rate ($F_{4,70} = 9.10$; $P < 0.0001$). Similar trends were recorded for all of the other parameters. In general, increased female parasitoid density beyond 5 wasps did not significantly improve the parasitoid performance on clusters of 100-200 FAW eggs (Table 7). However, the sex ratio was significantly higher for the exotic female parasitoid's progeny at the highest mother density (20 wasps) ($F_{1,27} = 5.79$; $P = 0.023$).

Table 7. Total parasitism (mean ± SE), adult emergence (mean ± SE), emergence rate (mean ± SE) and sex ratio (mean ± SE) of two strains of *Telenomus remus* released at five different densities on clusters of 100-200 eggs of *Spodoptera frugiperda* at ambient temperatures.

Parameter	**Parasitoid strain**	**Parasitoid density**				
		1	**5**	**10**	**15**	**20**
Total parasitism	Local	50.93 ± 8.17 Aa	107.2 ± 10.94 Aa	101.21 ± 7.24 Aa	113.85 ± 14.04 Aa	105.0 ± 15.32 Aa
	Exotic	39.42 ± 8.96 Ab	101.35 ± 13.54 Aa	95.5 ± 11.96 Aa	110.14 ± 4.53 Aa	93.85 ± 8.19 Aa
Adult emergence	Local	47.0 ± 8.51 Ab	105.86 ± 10.89 Aab	99.78 ± 7.40 Aa	111.28 ± 13.78 Aab	100.71 ± 15.31 Aab
	Exotic	38.28 ± 8.93 Ab	99.78 ± 13.40 Aa	94.28 ± 11.84 Aa	107.71 ± 4.59 Aa	89.92 ± 8.02 Aa
Emergence rate	Local	0.85 ± 0.07 Ab	0.98 ± 0.006 Aa	0.98 ± 0.007 Aab	0.97 ± 0.007 Aab	0.94 ± 0.01 Aab
	Exotic	0.95 ± 0.01 Aa	0.98 ± 0.008 Aa	0.98 ± 0.005 Aa	0.97 ± 0.007 Aa	0.95 ± 0.008 Aa
Sex ratio	Local	0.73 ± 0.06 Aa	0.63 ± 0.02 Aab	0.57 ± 0.02 Abc	0.47 ± 0.02 Acd	0.42 ± 0.03 Bd
	Exotic	0.80 ± 0.05 Aa	0.61 ± 0.02 Ab	0.53 ± 0.02 Abc	0.51 ± 0.01 Ac	0.52 ± 0.01 Ac

Means of local and exotic parasitoid strains at a given parasitoid density followed by different uppercase letters are significantly different (Tukey's tests at the 5% level). Means of a given parasitoid strain at various parasitoid densities followed by different lowercase letters are significantly different (Tukey's tests at the 5% level).

Discussion

The study of basic biological characteristics of parasitoid-host interaction, such as the functional response and life tables is of paramount importance. It can contribute to increased knowledge on the potential of the parasitoid to regulate pest population dynamics and it can give a basis for decision support for choices between strains or species of natural enemies.

In this study, the biological parameters of *T. remus*, namely, the oviposition time, the post-oviposition time, the female longevity, the lifetime fecundity and the adult emergence rate were generally statistically comparable between local and exotic strains under all experimental temperatures. However, the temperature negatively affected the egg-adult time for both local and exotic parasitoid strains. This is typical to poikilothermic organisms characterized by shortened developmental time in warmer temperatures. The results are consistent with other findings where the egg-to-adult period of *T. remus* on four *Spodoptera* species was inversely proportional to the increase in temperature (Krechemer & Foerster, 2015; Pomari et al., 2012). The life table statistics in the present study closely mirror other reports (Oktaviani et al., 2022; Sari et al., 2020). There was no temperature effect on adult emergence rate of either of the experimental parasitoid strains of *T. remus*. However, regarding mass rearing for augmentative biocontrol using *T. remus* against *S. frugiperda*, previous works have shown that the highest emergence rate of the parasitoid occurred when eggs were stored at 15°C for less than 9 days (Salazar-Mendoza et al., 2020). The present investigation discovered that the local strain performed better and was factually superior to the exotic strain at higher temperatures. This concurs to the assumption that in the African context, it would be scientifically sound to introduce the exotic strain of *T. remus* in environments with cool weather only.

Telenomus remus has the ability to parasitize both superposed and inner layers of the egg mass. It is well documented to have high fecundity, dispersal and host search capabilities underlining its potential for augmentative biological control programs on eggs of *Spodoptera* spp. (Colmenarez et al., 2022). Both strains of *T. remus* exhibited the type II functional response under all tested temperatures (25, 28 and 31 °C), which agrees with (Carneiro et al., 2010), thus concurring on the efficiency of the two strains. The fact that the local strain was most efficient at 31 °C is more evidence that it best fits the local environmental conditions, since warmer temperatures are the most frequent. This agrees with earlier conclusions in places with similar ecological parameters (Bueno & van Lenteren, 2001; Idrissou et al., 2020). However, *T. remus* in Africa might have arrived serendipitously much earlier and became better adapted than the strain from the Americas although both strains seem to have originated around Papua New Guinea. Besides, the fact that the most efficient egg parasitoid for FAW is of Asian origin while the pest itself is of American origin supports the high adaptability nature of *T. remus*. This is also an indication that it's likely that the exotic strain from the Americas if introduced will become well adapted to Africa in the future.

Higher parasitoid density did not improve the attack rate of *T. remus* for the local or exotic strains, indicating that there is a threshold beyond which it is unnecessary to utilize the addition of females for field release programs. This corroborates previous studies showing the same trend of parasitism stabilization despite the increase in density of *T. remus* (Salazar-Mendoza et al., 2020). The relative decline in parasitism rate with higher parasitoid density may be a result of increased competition or trade-offs on limited food source for offspring.

To conclude, temperatures ranging from 25 to 31 °C are suitable for the development and survival of both the local and exotic strains of *T. remus*. The same temperature range is the prevailing range in Benin. Both the local and the exotic strains of *T. remus* can be successful in Benin. Although it can be judged unnecessary to import and release *T. remus*, it may still be useful to opportunistically recruit exotic breeds to complement the action of local counterparts during colder periods. However, as this was a laboratory investigation, field experiments are required to support cost-effective decisions.

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Paper IV

Assessment of three release methods of *Telenomus remus* to control *Spodoptera frugiperda* in southern Benin: field cage experiments

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Abstract

The outcomes from the invasion and initial outbreaks of the fall armyworm (FAW) *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae) into Africa was ranked as the top priority biorisk to be mitigated in several African countries including Benin. Building on experiences with biological control of FAW in the Americas, diverse parasitoid release frequency and methods were suggested for adaptation to the African context. The present study aimed at comparing three parasitoid release methods under field cages for evaluating the performance of the locally recruited *Telenomus remus* strain in Benin. The experiment was arranged in a randomized complete block design, whereby 1) parasitoids were released weekly until maize tasseling stage; 2) parasitoids were released three times at 3-day intervals; 3) parasitoids were released at V4 and V10 stages of maize growth. The latter parasitoid release method proved to be statistically equal to the two other more demanding methods in terms of the number of parasitoids needed. This study is a pilot demonstration that minimal parasitoid release frequency can be cost-effective in the African context.

Keywords: Fall armyworm, egg parasitoid, parasitoid release, field efficiency, augmentation biocontrol

Introduction

The invasion and initial outbreaks of the fall armyworm (FAW) *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae) into Africa was ranked as the top priority biorisk in several countries including Benin. FAW does not undergo diapause and can cause extensive damage on several vegetable and other food crop species year-round (Day et al., 2017; Montezano et al., 2018; Pogue, 2002). Losses can particularly be devastating on maize - the prime host of FAW - which is the source of livelihood for more than 300 million people in sub-Saharan Africa (Houngbo et al., 2020). *Spodoptera frugiperda* became the most economically important insect pest on the continent in a very short time after the initial outbreaks (Day et al., 2017; Eschen et al., 2021).

Efforts to design and deploy sustainable integrated pest management strategies against FAW were highly contingent on the availability and accessibility of control options. The most used control methods were chemical, agroecological management (i.e. planting timing and habitat manipulations including intercropping and crop rotation) and farmer practices until the discovery of the egg parasitoid *Telenomus remus* Nixon (Hymenoptera: Scelionidae) adapting to the pest in Benin and in seven other African countries (Tepa-Yotto et al., 2022). These findings opened up opportunities for augmentative and conservation biological control (Kenis et al., 2019). *Telenomus remus* origin is reported to be Papua New Guinea and the peninsular Malaysia (Wengrat et al., 2021). The parasitoid is reported in several papers to be an efficient egg parasitoid of Lepidoptera insect pests and particularly those of the genus *Spodoptera* (Noctuidae) including *S. frugiperda* (Cave, 2000; Colmenarez et al., 2022).

It has now been well documented that the egg parasitoid *T. remus* has adapted to FAW in Benin (Agboyi et al., 2020; Winsou et al., 2022) and elsewhere on the African continent (Agboyi et al., 2021; Laminou et al., 2020; Sisay et al., 2019), raising hopes that it could become the object of augmentative releases. Building on experiences with biological control of FAW in the Americas (Bueno & van Lenteren, 2001), diverse parasitoid release frequency and methods were suggested for adaptation to the African context (Cruz et al., 2018; Pomari et al., 2013; Tefera et al., 2019). Given the emergency, those methods were sporadically applied without prior assessment of their effectiveness and success prerequisites. The present study was proposed to provide a scientific basis for recommending the most efficient release method, by examining the performance of the locally recruited *T. remus* strain in Benin; testing three parasitoid release frequency schemes through field cage experiments. Therefore, we hypothesized that three parasitoid release methods of the local strain of *T. remus* would be equally effective.

Materials and methods

Insects used

The fall armyworm cohorts used in this experiment were reared from larvae collected on maize fields in southern Benin. The insects were screened for pure cultures establishment and mass-reared under laboratory conditions. A routine mass rearing protocol for FAW was developed at the Biorisk Management Facility (BIMAF) laboratory hosted by the International Institute of Tropical Agriculture (IITA-Benin station) (Winsou et al., unpublished data). The rearing conditions were 26 ± 1 °C temperature, 65 ± 5% relative humidity and 12 hours photoperiod. FAW larvae were fed sprouting maize (variety EVDT) and raised to the pupal and adult stages. The sprouting maize was obtained by soaking maize kernels to break the dormancy and leaving them to germinate in a tray under laboratory conditions. The food was surface sterilized with a 5% antimicrobial sodium hypochlorite solution and rinsed several times with abundant distilled water to wash off the bleach before feeding the FAW caterpillars. The pupae were disinfected similarly and placed in oviposition boxes (18cm diameter; 10cm height) covered with cotton gauze until the moths emerged. The hatching moths were allowed to mate in the oviposition boxes and fed with a 10% honey solution imbibed from capsules of cotton wool. After copulation, FAW females laid eggs and egg masses on the cotton gauze cover and on the oviposition box walls approximately two days post-emergence. Eggs and egg masses were collected every two days and incubated in new rearing boxes containing sterile sprouting maize.

The local strain of *T. remus* used in this study was collected on FAW egg masses in maize fields in Athieme Assedji (6.853333°N, 1.888333°E), in southern Benin. The parasitized egg masses were brought to the laboratory for adult parasitoid emergence and parasitoid mass production. The initial cohort was maintained on FAW egg masses for experimental purpose. In brief, fresh egg masses were sourced from FAW rearing as described above. They were carefully cut with the sterilized gauze on which they were laid and further confined to Petri dishes (9cm diameter, 1.4cm height) with ten female *T. remus* for oviposition. The parasitoids were offered FAW eggs ad libitum and mass reared to produce enough offspring for the experiments. The adults were fed using droplets of honey deposited on the lid of the Petri dish. *Telenomus remus* was mass produced in the laboratory at 26 ± 1°C, 60 ± 5% relative humidity and 12 hours light/dark photophase.

Study site and planting under field cages

Rainfall in southern Benin has a bimodal pattern (Mar–Jul and Sep–Nov) which offers two distinct growing windows, the first being the major rainy season and the second the minor. All experiments were conducted at the International Institute of Tropical Agriculture (IITA-Benin) station, Cotonou, Benin (6.417500°N, 2.331500°E) during both major and minor seasons. The commonly grown extra-early maize variety TZE was sown on May 17th, 2021 (major season) and on September 17th, 2021 (minor season) at a spacing of 80cm between rows and 40cm between plants.

All the plots were maintained under field cages (2m length, 2m width and 2m height) covered with muslin cloth. Each plot had a total of 15 maize plants. The experiments were conducted throughout the maize growing season and data collection were done during the early and late whorl stages (McGrath et al., 2018). Nitrogen, phosphorus and potassium ($N_{15}P_{15}K_{15}$)-fertilizer was applied at the dosage equivalent to 100 kg per hectare two weeks after maize emergence and just after the first weeding. Urea (46% nitrogen) was applied at a dosage equivalent to 50 kg per hectare two weeks after the first fertilizer application. In total three weedings were done every two to four weeks depending on weed density.

Artificial infestation of maize plants

At the 4-leaf vegetative stage (V4) of the maize plants, approximately 7 days post-emergence of maize plants, five couples of newly emerged FAW adults were selected for artificial infestation in the experimental cages. The plants were carefully inspected the day before the first release of the moths to confirm that they had not been accidentally infested. The moths were released using a plastic rearing box (18cm diameter, 10cm height) containing the 10 insects confined for mating, which was subsequently opened at the center of the field cage to allow the female moths to oviposit on the plants. The pest release in each field cage was renewed every 10 days throughout the experiments. The moths were released one day before the release of the parasitoids. The estimated egg load per female is 1500 to 2000 eggs in masses of 50 to 200 eggs each during their lifetime. Fall armyworm release was done in the afternoons at 4:00pm to avoid direct exposure to sunlight and each release was postponed to the following day in the event that weather forecasts predicted night rains.

Experimental procedure and release methods

Two selected parasitoid release methods (treatments 1 and 2) were proposed to kill FAW eggs in the African context (Cruz et al., 2018; Tefera et al., 2019) without previous assessment of their effectiveness. These were compared to a third method (Pomari et al., 2013) tested earlier in the Americas. Therefore, four treatments were set in a randomized complete block design with four replicates; treatment 1) weekly parasitoid release until the maize tasseling stage (Tefera et al., 2019); treatment 2) three consecutive parasitoid releases at three day intervals (Cruz et al., 2018); treatment 3) parasitoid release at V4 and V10 stages of maize growth (Pomari et al., 2013); treatment 4) only FAW adults were released in control field cages and eggs on infested plants were monitored to verify cage mesh tightness and isolation from external organisms. The four treatments within blocks were separated by 20m from each other while there was 30m separation between each of the four blocks. Clusters of fifty *T. remus*-parasitized eggs of FAW were glued on cardboard (6cm x 2.5cm) (Tefera et al., 2019) to test the three parasitoid release methods. The parasitized egg cards were hung on maize plants (one egg card per treatment) 11 days post-exposure to *T. remus* females, i.e. the day before expected adult parasitoid emergence. The parasitoid release commenced 7 days after emergence of the maize plants, approximately at the 4-leaf vegetative stage (V4), and until tasseling. The parasitoids were released in the afternoons at 4:00pm, which is the same time as the moth release. The first release of *T. remus* was done on May 30th, 2021 and on September 30th, 2021 during the major and the minor season, respectively. The last release of the parasitoid was completed on July 19th, 2021 (major season) and on November 19th, 2021 (minor season).

Data collection and analysis

After parasitoid release, all egg masses were collected each two days and three times a week from all the 15 plants within every field cage and kept separately in transparent labelled plastic bags. The collected egg masses were conditioned separately in aerated Petri dishes, carried to the laboratory and monitored at 26 ± 1°C. These eggs were followed daily to quantify parasitism rates. The parasitized eggs were checked for successful parasitism under a stereomicroscope (Chen et al., 2021) and monitored daily until adult parasitoid emergence. No parasitism occurred on control plants and the data were not included in further analyses.

To compare the three parasitoid release methods, four parameters were considered in data analyses for both the major and the minor seasons. These parameters include: the number of FAW eggs parasitized by *T. remus*; the number of adult parasitoids which emerged; the percentage of FAW eggs parasitized by *T. remus*; and the percentage of adult parasitoids emerged.

The number of FAW egg masses parasitized by *T. remus* and of adult parasitoids emerged were log-transformed before analysis to meet the assumptions of normality and equal variance. The data were then analyzed using a linear model analysis of variance (ANOVA) type II sum of squares with parasitoid release method as the fixed effect factor, and maize growth period as a categorical variable. Comparisons were done over two registration periods matching two maize growth periods: early whorl stage (0-21 days) and late whorl stage (22-49 days) (McGrath et al., 2018). All observation dates were grouped into the two periods. Tukey's post hoc tests at the 5% significance level was used to examine differences among the groups, followed by pairwise comparisons (R statistical software; R Core Team, 2012).

The parasitism rate was calculated by dividing the total number of parasitized eggs by the total number of eggs counted within an egg mass. The percentage of emerging adult parasitoids was computed as the ratio of the total number of hatching adults to the total number of parasitized eggs. The data were subjected to angular transformation to meet the assumptions of normality and variance homogeneity. The arcsine square-root transformed data were then exposed to two-way ANOVA for testing parasitoid release method as the fixed effect factor, and maize growth period as a categorical variable. Mean separation was performed using Tukey's post hoc tests at the 5% significance level (R statistical software (R Core Team, 2012).

Results

Major maize cropping season

The number of FAW eggs parasitized by *T. remus* during the major season was generally higher with parasitoid release in treatment 3 (release at V4 and V10). However, there were no statistical differences between the three methods (Table 1). Combining all the parasitoid release methods, parasitism was statistically significantly higher during the early whorl stage of the maize (0-21 days) during the major season. During the major season, there was a significant interaction between the parasitoid release method and the maize growth. The highest numbers of FAW eggs parasitized were observed during both the early whorl stage with treatment 1 (weekly releases), and the late whorl stage with treatment 3 (release at V4 and V10) (Figure 1). The same trends occurred for the number of adult parasitoids emerged, except that there was no difference between the two maize phenological stages examined (Table 1; Figure 1). The percentage egg parasitism and adult parasitoid emergence were the same as those presented above, the only exception being that there was a significant difference between the two maize stages registered for the percent adult parasitoids emerging (Table 1; Figures 1 & 2). The statistically significant highest number of FAW eggs parasitized (25.35 ± 8.37) and percent adult parasitoid emergence (28.64 ± 4.80%) were recorded for parasitoid release in treatment 3, during the late whorl stage of maize phenology (22-49 days), and for parasitoid release in treatment 1, during the early whorl stage of maize phenology (0-21 days), respectively (Figures 1A & 2B).

Table 1. Effect of the release method of *T. remus* females* and maize growth ** on the number (log-transformed) and percentage (arcsine square-root transformed) of FAW eggs parasitized and adult parasitoid emergence; ANOVA results of the major season.

Source	df	SS	MS	*F*	*P*
Number of FAW eggs parasitized					
Release method	2	2.510	1.254	2.506	0.082
Stage	1	2.379	2.378	4.751	0.029
Release method x Stage	2	7.452	3.726	7.442	0.0006
Residuals	398	199.257	0.500		
Number of adult parasitoids emerged					
Release method	2	0.410	0.205	0.450	0.637
Stage	1	1.361	1.360	2.986	0.084
Release method x Stage	2	7.509	3.754	8.239	0.0003
Residuals	398	181.366	0.455		
Percentage of FAW eggs parasitized					
Release method	2	0.170	0.085	0.259	0.771
Stage	1	4.365	4.365	13.319	0.0002
Release method x Stage	2	4.100	2.050	6.255	0.002
Residuals	398	130.443	0.327		
Percentage of adult parasitoids emerged					
Release method	2	0.019	0.009	0.037	0.963
Stage	1	3.151	3.151	12.201	0.0005
Release method x Stage	2	3.137	1.568	6.073	0.002
Residuals	398	102.793	0.258		

* Parasitoid release started at the 4-leaf vegetative stage (V4) of maize phenology, approximately one week after emergence of maize plants: treatment 1 (weekly releases); treatment 2 (three consecutive releases); treatment 3 (release at V4 and V10).

** Maize growth stages [early whorl stage (0-21 days) and late whorl stage (22-49 days)].

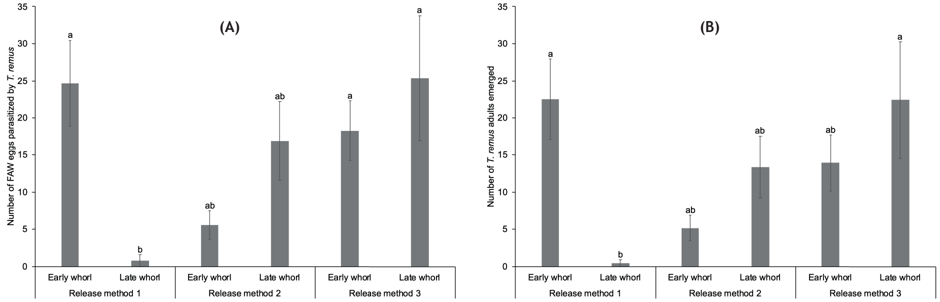

Figure 1. (A) Mean number of FAW eggs parasitized by *T. remus* and (B) mean number of adult parasitoids emerged (± SE) for three parasitoid release methods at two maize growth stages (early whorl and late whorl stages) during the major season. Parasitoid release started at the 4-leaf vegetative stage (V4) of maize phenology, approximately one week after emergence of maize plants: treatment 1 (weekly releases); treatment 2 (three consecutive releases); treatment 3 (release at V4 and V10). Error bars denote standard errors. Means with same letters are not significantly different (Tukey's tests at the 5% level).

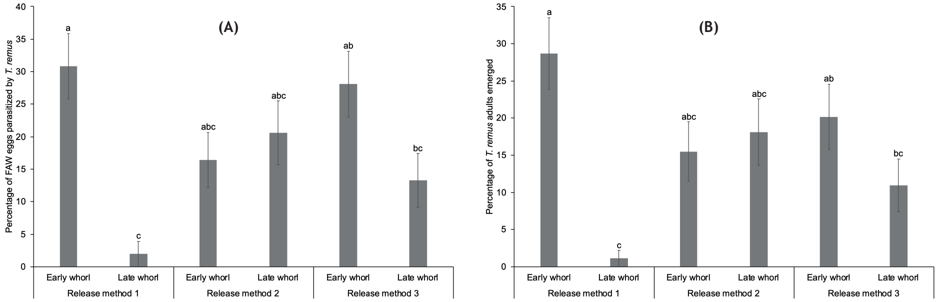

Figure 2. (A) Average percentage of FAW eggs parasitized by *T. remus* and (B) average percentage of adult parasitoids emerged (± SE) for three parasitoid release methods and at two maize growth (early whorl and late whorl stages) during the major season. Parasitoid release started at the 4-leaf vegetative stage (V4) of maize phenology, approximately one week after

emergence of maize plants: treatment 1 (weekly releases); treatment 2 (three consecutive releases); treatment 3 (release at V4 and V10). Error bars denote standard errors. Means with same letters are not significantly different (Tukey's tests at the 5% level).

Minor maize cropping season

The parasitoid release method in treatment 1 (weekly releases), yielded a relatively higher parasitism rate during the minor season (Table 2; Figures 3 & 4). In general, the parasitism was significantly higher during the late whorl stage (22-49 days). The highest number of FAW eggs parasitized (54.16 ± 10.70) and percent adult parasitoid emergence (36.49 ± 5.86%) were most evident for treatment 1 during the late whorl stage of maize phenology (22-49 days) (Figures 3A & 4B).

Table 2. Effect of the release method of *T. remus* females* and maize growth stage** on the number (log-transformed) and percentage (arcsine square-root transformed) of FAW eggs parasitized and adult parasitoid emergence; ANOVA results of the minor season.

Source	df	SS	MS	*F*	*P*
Number of FAW eggs parasitized					
Release method	2	2.576	1.288	2.234	0.108
Stage	1	10.376	10.375	17.999	2.85×10^{-5}
Release method x Stage	2	5.783	2.891	5.016	0.007
Residuals	339	195.415	0.576		
Number of adult parasitoids emerged					
Release method	2	2.370	1.185	2.162	0.116
Stage	1	10.610	10.610	19.363	1.45×10^{-5}
Release method x Stage	2	5.054	2.526	4.611	0.010
Residuals	339	185.755	0.547		
Percentage of FAW eggs parasitized					
Release method	2	0.641	0.320	0.854	0.426
Stage	1	5.227	5.226	13.935	0.0002
Release method x Stage	2	2.784	1.392	3.712	0.025
Residuals	339	127.142	0.375		

Table 2. *Cont.*

Source	df	SS	MS	*F*	*P*
Percentage of adult parasitoids emerged					
Release method	2	0.573	0.286	0.877	0.416
Stage	1	4.079	4.078	12.485	0.0004
Release method x Stage	2	2.213	1.106	3.387	0.034
Residuals	339	110.742	0.326		

* Parasitoid release started at the 4-leaf vegetative stage (V4) of maize phenology, approximately one week after emergence of maize plants: treatment 1 (weekly releases); treatment 2 (three consecutive releases); treatment 3 (release at V4 and V10).

** Maize growth stages [early whorl stage (0-21 days) and late whorl stage (22-49 days)].

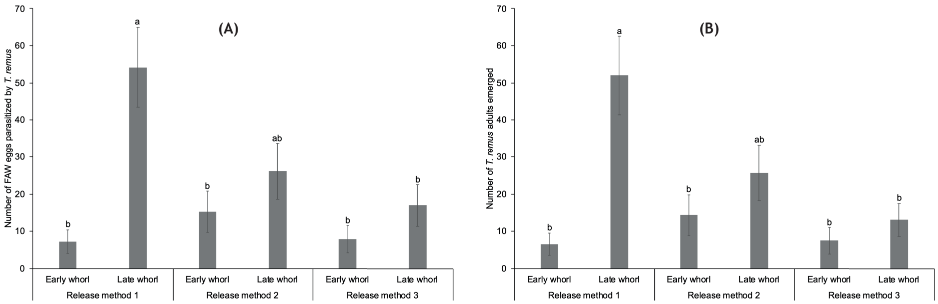

Figure 3. (A) Mean number of FAW eggs parasitized by *T. remus* and (B) mean number of adult parasitoids emerged (± SE) for three parasitoid release methods at two maize growth periods (early whorl and late whorl stages) during the minor season. Parasitoid release started at the 4-leaf vegetative stage (V4) of maize phenology, approximately one week after emergence of maize plants: treatment 1 (weekly releases); treatment 2 (three consecutive releases); treatment 3 (release at V4 and V10). Error bars denote standard errors. Means with same letters are not significantly different (Tukey's tests at the 5% level).

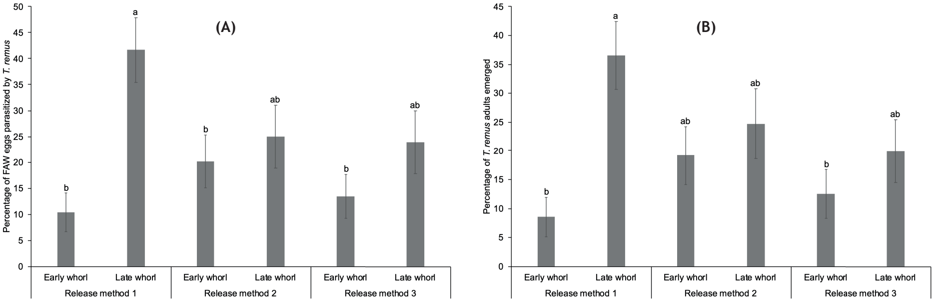

Figure 4. (A) Average percentage of FAW eggs parasitized by *T. remus* and (B) average percentage of adult parasitoids emerged (± SE) for three parasitoid field cage release methods at two maize growth periods (early whorl and late whorl stages) during the minor season. Parasitoid release started at the 4-leaf vegetative stage (V4) of maize phenology, approximately one week after emergence of maize plants: treatment 1 (weekly releases); treatment 2 (three consecutive releases); treatment 3 (release at V4 and V10). Error bars denote standard errors. Means with same letters are not significantly different (Tukey's tests at the 5% level).

Synthesis of both seasons

The three parasitoid release methods tested were statistically similar in terms of performance. None of the experiments revealed significant differences between the methods in both cropping seasons (major and minor). However, treatment 1 (weekly releases) was the most efficient in the minor season, which was not the case during the major season where treatment 3 (release at V4 and V10) was the best. The success of the parasitoid release methods differed throughout the maize development cycle, early whorl stage (0-21 days) and late whorl stage (22-49 days). Overall, the parasitism rate improved during the late whorl stage (Figures 1-4), except that an opposite trend was observed for treatment 1 (weekly releases), during the major season (Figure 1). Both the parasitism and parasitoid adult emergence rates were higher during the minor season compared to the major season, 1.3 to 5.3-fold and 1.2 to 7.8-fold respectively. For all parameters measured there were significant interactions between the parasitoid release method and the maize growth period.

Discussion

Augmentative biological control using egg and egg-larval parasitoids might be the most practical approach against FAW, compared to larval parasitoids because of the cannibalistic behavior of the pest in its larval stage (Bueno & van Lenteren, 2001). Nevertheless, mass-production of parasitoids for inundative/inoculative release might not be an easy task, and it requires specialized facilities (Castellanos et al., 2019). Our observation revealed that none of the parasitoid release methods was statistically significantly superior with regard to parasitism efficiency. Treatment 1 (weekly releases), was the most demanding in terms of the number of wasps required, 1400 parasitoids released during the whole experiment, compared to the others, 600 for treatment 2 (three consecutive releases) and 400 wasps for treatment 3 (release V4 and V10). Treatment 3 (release V4 and V10) was less arduous. While the release of a large number of individuals instead of rationale deployment may provide a quick knockdown effect on the pest, our study and previous observations suggest that it is unnecessary (Winsou et al., unpublished data). Some literature suggests that release densities/dispersal arrangements of *T. remus* should be 20 to 70 release points per hectare of the egg parasitoid at a density of 100,000 to 120,000 individuals per hectare (Cruz et al., 2016; Salazar-Mendoza et al., 2020; Tefera et al., 2019). Experience with successful mass production and release of *T. remus* for more than 40 years in Venezuela gave an estimate of the cost of its production per unit of 1000 wasps in June 2021 to be USD 1.89 (Colmenarez et al., 2022). The total cost per hectare was thus USD 15.1, based on a release rate of 8000 *T. remus* individuals per hectare. Although mass production costs can appear affordable for largescale farmers, this might not be the case for the majority of small-scale farmers in Africa. While chemical control methods are cheaper (approximately 10 US dollars per hectare), their use can be associated with adverse health and environmental effects and costs. In addition, parasitoid production requires some technical handling skills and facilities. Most African countries lack the necessary rearing capacity. This could be covered by the private sector engagement, but there is not yet such industry in West Africa. Therefore, reducing the frequency of the releases of parasitoids will reduce costs while achieving comparable results in terms of pest population reduction.

In Ghana and Niger (West Africa), some field release experiments have been conducted using the locally recruited *T. remus* against FAW (Agboyi et al., 2021; Laminou et al., 2020) but they did not investigate different release methods. In the present study, the parasitization rate of *T. remus* improved during the late whorl stage with 3 consecutive releases or release at V4 and V10 during the major season. Furthermore, there were significant interactions between the parasitoid release method and maize growth, which indicates that the effectiveness of the parasitoid release method varies across the plant phenology. In general, the attack rate and the parasitoid population increased later during the crop cycle, particularly during the minor season. There is a need to drastically curb the incidence of FAW during the early whorl stage particularly in instances of severe outbreaks when the pest can cause critical loss of the whole crop (Tepa-Yotto et al., 2021). Under severe pest pressure, the early whorl stage is the most sensitive phenological stage and requires fast-acting control interventions. This can only be achieved in an integrated pest management approach where effective biopesticides or low-toxicity chemical insecticides can complement augmentative releases without negatively affecting the activity of the parasitoids. However, maize plants have intrinsic recovery potential under pressing biotic and abiotic stresses because of their silica content (Nagaratna et al., 2022) and they can also develop defense mechanisms if they have enough rain and sufficient nutrients. Therefore, in cases of low to moderate FAW infestation, augmentative releases with *T. remus* may be an effective standalone option. Interestingly, parasitism rates were significantly higher in the early whorl stage during the major season, especially for treatment 1 (weekly releases). The findings suggest that the parasitism rate might be higher at the beginning of the major rainy season following the long drought period. The findings also suggest that combinations of parasitoid release frequencies could be explored to enhance augmentation effectiveness. As an example, weekly parasitoid releases (during the early whorl stage) can effectively complement the action of releases at V4 and V10 (during the late whorl stage).

The parasitism and parasitoid adult emergence rates were 1.3 to 5.3-fold and 1.2 to 7.8-fold higher during the minor season compared to the major season. These results are comparable to earlier findings in open fields in Ghana (Agboyi et al., 2021). However, a carry-over parasitoid population from the major season can colonize open fields much earlier during the minor season, which makes the situation different as compared to this study. Therefore, arguments focused on climatic conditions and plant phenology are at best speculative. Explanations for the increased parasitoid efficiency during the minor season should be explored in further investigations both under cages and open field.

To conclude, this study demonstrates that minimal parasitoid release frequency can be cost-effective in the African context. The release of parasitoids at V4 and V10 stages of maize growth, proved to be as efficient as the two other methods, both of which are more demanding in terms of the number of parasitoids needed, and consequently, economic resources. However, as the current investigation was done under field cages, studies should be done in an open field situation before we can conclude that this will hold true in practice.

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