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COLLEGE OF BASIC AND APPLIED SCIENCES

DIVERSITY AND SOME ASPECTS OF THE BIOECOLOGY OF APHIDS ON CABBAGE AND THEIR ASSOCIATION WITH A NOVEL NECROTIC DISEASE.

BY
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10513147

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DECLARATION

I hereby declare that this thesis is the original research work carried out by, Forchibe Ethelyn Echep for the award of Doctor of Philosophy (PhD) in Entomology at the African Regional Postgraduate Programme in Insect Science (ARPPIS) from the University of Ghana, Legon. All references to other peoples' work have been duly acknowledged and the thesis has not been submitted in part or whole for the award of a degree elsewhere.

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ABSTRACT

Agriculture serves as a significant source of livelihood for many small-scale farmers, who account for the greatest share of agricultural production. Of the numerous varieties of exotic vegetables grown in Ghana, cabbage (*Brassica oleracea* var. capitata) is widely grown to meet the high demand of the ever-growing urban market, due to the many health benefits associated with its consumption. However, the devastating impacts of pests such as aphids and diseases on cabbage have resulted in significant yield losses. Aphids achieve this fate by developing resistance to many insecticides by rendering them ineffective, heavy infestations, development of sooty mould and transmission of viral diseases. Management interventions have been largely dependent on calendar spray regimes, with little or no knowledge on pest species and bioecology. While these interventions are an integral component of an integrated pest management approach on cabbage, identifying pest species, understanding their bioecology is important in the development and success of these pest management interventions. The current study sought to understand the diversity and bioecology of aphids on cabbage, and their role in the aetiology of a novel necrotic disease in Ghana. Surveys were conducted from 2019 to 2020, in nine regions of Ghana across five agroecological zones, noted for the production of cabbage to determine aphid species associated with cabbage and their distribution. Their regional abundance and spatiotemporal variation across agroecological zones were also assessed. In each region, cabbage farms in three districts were selected for the survey. Sampling was done by randomly sampling 20 plants per farm and aphids were counted, and some collected into vials containing 70% alcohol for identification. Two aphid species were identified to occur on cabbage, i. e. *Lipaphis erysimi pseudobrassicae* and *Myzus persicae*, with the former being the more abundant in all the regions. The highest aphid distribution and abundance was recorded in the Southern part of the country. There was significant variation in the population of these aphids across the different agroecological zones, with the highest recorded in the Deciduous Forest and Coastal Savannah zones. Aphid abundance was influenced by agroecological zones, vegetative cover and climatic conditions. The distribution of *L. e. pseudobrassicae* and *M. persicae*, in Ghana could not be ascertained by manually sampling all cabbage growing regions, hence, the rationale for the use of species distribution models to understand their spatial patterns and the underlying contributing climatic factors to map their suitable habitats. Occurrence data collected during the survey was used to determine

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aphid spatial distribution. A tree-based supervised machine learning algorithm, Random Forest was used to infer the underlying contributing factors, and to map the habitat suitability of both aphid species. Temperature and isothermality were the most important weather variables that affected the distribution of *L. e. pseudobrassicae* and *M. persicae.* The Southern Ghana was most suitable for the occurrence of both aphid species. Species distribution models can inform researchers about particular ecological adaptations of aphids and other insect pests in targeted areas, which can optimize efficiency and cost in pest control programmes.

Aphid dynamics on crops is often influenced by climatic factors and their association with natural enemies. Experimental fields were setup in Kpong (Coastal Savannah agroecological zone) and Aseseeso (Deciduous Forest agroecological zone), and populations of *L. e. pseudobrassicae*, *M. persicae* and their natural enemies were monitored and recorded for five growing seasons (2019-2021). The mean population and infestation of *L. e. pseudobrassicae* were largest during the minor rainy seasons of 2019 and 2020 in the Deciduous Forest agroecological zone, and during the dry season of 2019 and minor rainy season of 2020 in the Coastal Savannah agroecological zone. Largest mean population and infestation for *M. persicae* were recorded during the minor season of 2019 and the dry season of 2020 in the Deciduous Forest zone and during the minor seasons of 2019 and 2020 of the Coastal Savannah agroecological zone. Significant differences in average aphid count per leaf within and between seasons were recorded for both agroecological zones. The Deciduous Forest zone showed high natural enemy diversity. *Lipaphis erysimi pseudobrassicae* and *M. persicae* populations showed significant negative correlation with temperature in both agroecological zones, while significant positive correlation with precipitation and relative humidity were recorded with *M. persicae* in the Coastal Savannah zone. This study provides information on aphid incidence across different seasons and agroecological zones, peak aphid activity, in relation to climatic factors and natural enemies. To understand the growth and development of both aphid species on commonly grown cabbage varieties, the biological and population growth parameters were studied on three cabbage varieties (Oxylus, Fortune and Leader cross). The study was carried out in a screen house under ambient conditions at 30 ± 1ºC and 75 ± 5%RH from September to November, 2020. The preadult developmental period, survival rates, longevity, reproduction, and life table parameters were evaluated following the female age-specific life table. There were significant differences in the nymphal developmental time, longevity and fecundity on the different cabbage varieties for both aphid species. The highest

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population growth parameters; net reproductive rate (R_0), intrinsic rate of increase (r), and finite rate of increase (λ) were recorded on oxylus variety for both *L. e. pseudobrassicae* (R_0 = 47.05 offspring, r = 0.314 d^{-1}, λ = 1.368 d^{-1}) and *Myzus persicae* (R_0 = 23.75 offspring, r = 0.249 d^{-1}, λ = 1.282 d^{-1}). The lowest were recorded on leader cross variety (R_0 = 6.46 offspring, r = 0.174 d^{-1}, λ = 1.191 d^{-1}) for *L. e pseudobrassicae*, and Fortune (R_0 = 2.94 offspring, r = 0.088 d^{-1}, λ = 1.092 d^{-1}) for *M. persicae*. The results suggests that leader cross is a less suitable host for *L. e pseudobrassicae*, and fortune for *M. persicae*, and thus, should be considered as potential sources of host plant resistance in breeding programmes. Aphids were found associated with a necrotic disease on cabbage suspected to be of viral aetiology.

To understand the role of *L. e. pseudobrassicae*, and *M. persicae* in the transmission and expression of the necrotic disease, diseased leaves were subjected to RT-PCR to identify the causal pathogen, after which transmission experiments were conducted following Koch's postulate to confirm identified pathogen. A cabbage field was setup at Kpong to study aphid/disease interaction. Cucumber Mosaic Virus (CMV-GH) was identified to be associated with the observed disease on cabbage but could not be confirmed as the causal pathogen, following failed transmission experiments to demonstrate Koch's postulates. A strong positive relationship between aphid occurrence and disease onset were recorded. These findings are consistent with a role for *L. e. pseudobrassicae* and *M. persicae* in the transmission of the novel necrotic disease of cabbage. Farmers' perception of aphids as pests of cabbage, their associated damage and management in Southern Ghana was assessed. A total of 121 farmers were interviewed in person using semi-structured questionnaires. Farmers had a good knowledge on aphid dynamics; aphid occurrence and seasonal variation, their associated damage and management, and consequently viewed aphid as one of the most damaging pests of cabbage. Seven varieties of cabbage were cultivated by farmers which included oxylus, fortune, kk cross, super cross, santa, Copenhagen market and leader cross, but over 80% of them cultivated Oxylus variety, which was known as 'farmers' choice'. Farmers employed a wide range of control measure particularly pesticide use, sometimes rotating synthetic with biological pesticides, and other times, mixing a 'cocktail' of different pesticides. The findings confirm indigenous knowledge, which is relevant in pest management/breeding programmes, and in the development of new technologies and adaptive strategies to changing food systems.

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DEDICATION

To my parents, Mr. Njoh Stephen Forchibe and Mrs. Forchibe Vivian Mawom, my lovely son, Ian-Jaiden Kojofoah Forchibe and my sister Forchibe Harriet Efong,

Your support and Love throughout this journey have been insuperable.

Thank you, I Love you forever.



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INTEGRI PROCEDAMUS

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LIST OF ABBREVIATIONS

FAO - Food and Agriculture Organization of the United Nations

USDA – United States Department of Agriculture

MoFA – Ministry of Agriculture

AEZ - Agroecological zone

PCR – Polymerase chain reaction

CMV – *Cucumber mosaic virus*

TuMV – *Turnip mosaic virus*

BWYV – *Beet western yellows virus*

CaMV – *Cauliflower mosaic virus*

RT-PCR - Reverse transcription-polymerase chain reaction

RNA – Ribonucliec acid

gDNA – genomic deoxyribonucleic acid

cDNA – complementary deoxyribonucleic acid

dNTP - deoxy-nucleoside triphosphates

SDM - Species distribution modelling

RF – Random Forest

ANN – Average nearest neighbour

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CHAPTER 1

1.0 GENERAL INTRODUCTION

1.1 Background

Cabbage, *Brassica oleracea* var. *capitata* L. is a crop belonging to the family Brassicaceae, and crops of this family are of great economic importance worldwide (Chiang *et al*., 1993; Bhatia *et al.*, 2011). Its cultivation provides livelihood for both rural and urban dwellers (Abbey and Manso, 2004; Owusu-Boateng and Amuzu, 2013; Amoabeng *et al.*, 2017). It is an exotic vegetable known native to Southern Europe and the Mediterranean regions (FAO, 2000). It is an important source of food and nutrients globally and has been listed as one of the most important green vegetables worldwide (FAO, 2020). It is grown throughout the world in more than 90 countries including the tropics (Sawant *et al.*, 2010; FAO Stats, 2011). This vegetable has valuable nutrients such as vitamins, dietary fiber, calcium, fatty acids; and it is being used across different cultures around the world for medicinal purposes (Ahuja *et al*., 2013). For example, the antioxidant property of cabbage is known to fight cancer (Liu, 2004; Tajalli *et al*., 2020).

Several varieties of cabbage are grown around the world and are known to be well adapted to a wide range of climatic conditions (Shinohara, 1965). In Ghana, the major varieties grown include Oxylus, Fortune, KK cross, Copenhagen market, Super cross, Santa F1, and Vantar F1 hybrid of which oxylus and fortune are often intensively grown to meet the ever-growing demand (Adenka *et al.*, 2020). It is typically eaten raw (in salads, sandwiches, and hamburgers) or cooked, in sauces (Baidoo *et al.*, 2012). Cabbage is noted as one of the major staple vegetables grown in Ghana (GhanaVeg, 2014).

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Cabbage is susceptible to attack by insect pests and diseases during its cultivation, which causes a significant reduction in yield and quality, thereby affecting its market value (Zehnder *et al.,* 1997; Fening *et al.,* 2013). Adenka *et al*. (2021) reported up to 100% yield loss of cabbage, attributed to insect pests and diseases. Of the different insect pests that attack cabbage, aphids are considered as major pests of economic importance and great concern because in addition to the physical damage they cause, they are also vectors of plant viruses such as *Cauliflower mosaic virus* (CaMV), *Cucumber mosaic virus* (CMV), and *Turnip mosaic virus* (TuMV), which cause huge losses in Brassica crop production worldwide (Rohilla and Kumar, 1991; Munthali *et al.,* 2004), thus drawing attention as a major pest of economic importance.

1.2 Justification

Aphids are 'small, pear-shaped, delicate insects with soft, fragile bodies', belonging to the superfamily Aphidoidea (Blackman and Eastop, 2000). They may be winged or wingless, with their nymphs resembling the adults, and extensive colour variation even within a single colony, a phenomenon known as colour polymorphism. (Blackman and Eastop, 2000; VanEmden and Harrington, 2007). Colour polymorphism often affects the fitness of aphids and in some cases, affects their susceptibility to an array of natural enemies (Caillaud and Losey, 2010). Aphids cause severe damage to crops and their ability to inflict direct and indirect damage on crops categorises them as one of the most detrimental insect pests on cultivated plants (Sorenson, 2009). Direct plant damage includes stunting, distortion, yellowing, and wilting, usually resulting from sucking sap from infested plants (Hughes, 1963; Aslam *et al.*, 2011). Indirect damage includes dark sooty mold formed from fungus growth on honeydew excretion, the transmission of plant viruses that results in diseases, and plant deformations because of toxic saliva secretions (Blackman and Eastop,

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2000). Apart from preventing adequate photosynthesis, sooty mold and honeydew also reduce the aesthetic value and marketability of crops (Fening *et al.,* 2013; Forchibe *et al.,* 2017). The pest status of aphids is enhanced by their capability to reproduce fast, and adapt to new environments (Myburgh, 1993).

The major aphids of economic importance that occur on cabbage worldwide are the cabbage aphid, *Brevicoryne brassicae* (Linnaeus), (Hemiptera: Aphididae), the Indian mustard or false cabbage aphid; *Lipaphis erysimi pseudobrassicae* (Davis), and the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), (Liu and Sparks, 2001; Mpumi *et al*., 2020). *Brevicoryne brassicae* and *L. e. pseudobrassicae* are specialist aphids feeding solely on brassica crops, whereas *M. persicae* is a generalist, feeding on several crop families (Blande *et al*., 2004; Van Emden, 2007; Fening *et al*., 2016). In Ghana, *L. e. pseudobrassicae and M. persicae* have been reported to be the major aphids of economic importance on cabbage (Fening *et al.,* 2016; Forchibe *et al.,* 2017; Adenka *et al.,* 2021). *Lipaphis e. pseudobrassicae* was first recorded in Ghana in 2016, as one of the major aphids on cabbage, causing devastating losses often leading to little or no marketable cabbage heads (Fening *et al.,* 2016; Forchibe *et al.,* 2017). It is mostly found in association with *M. persicae,* an extremely polyphagous aphid feeding on a wide variety of plant species in over 40 different families (Eastop and Hille, 1976; Blackman and Eastop 2000). Its attack often leads to severe damages in associated plants and has been implicated in transmitting over 40 viruses to different crops (Blackman and Eastop, 2000). The cabbage aphid; *B. brassicae* (Linnaeus), has also been cited by many authors as occurring on cabbage in Ghana (Fening *et al*., 2014; Amoabeng *et al*., 2013; Ngosong *et al*., 2020), although it is known as a temperate and subtropical species. This therefore raises concerns about its occurrence and distribution in Ghana as they are sometimes mistaken for the false cabbage aphid, *L. e. pseudobrassicae* due to their similarity in appearance

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(Blackman and Eastop, 2000). Additionally, there are no detailed studies on aphids in Ghana to firmly attest that this species was present in the country. Thus, proper identification of aphid species on brassica crops in Ghana was essential for their subsequent control and implementation of quarantine measures.

Traditionally, morphological characteristics have been used to identify aphids (Van Emden and Harrington, 2007). However, due to their small sizes, the presence of immature stages, the presence of cryptic species, the similarity between species, and loss of key morphological characteristics after collection (e.g., damaged specimens), identification has become almost impossible (Foottit *et al*., 2008; Miller and Foottit, 2009; Lee *et al.,* 2011). Additionally, 'the presence of cryptic species in a genus that constitutes significant agricultural pests represents a serious gap in the management of aphid pest species' (Valenzuela *et al.,* 2009). The complexity of the life cycle of aphids which involves pathogenesis and high polymorphisms, causes different morphs to exist within one species, thus complicating species identification (Blackman and Eastop, 2000; Miller and Foottit, 2009). These further buttresses the need to clarify the species status of aphids found on cabbage in Ghana using molecular tools to confirm what has been already identified using morphological methods.

As vectors of plant viruses, aphid occurrence on crops is a major cause for concern. Studies has shown that aphids generally account for the transmission of up to 50 % of insect-vectored viruses worldwide (Brunt *et al.,* 1996; Nault, 1997; Hogenhout *et al.,* 2008). Their piercing-sucking mouthparts coupled with their high reproductive rates make them agents of permeating disease epidemics and spreading of plant viruses (Stafford *et al*., 2012). Viral transmission could be persistent, semi-persistent, or non-persistent depending on the viral pathogen (Brunt *et al.*, 1996). Cucumber mosaic virus (CMV) for example, is transmitted by *M. persicae* non-persistently, and

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is known as one of the most prevalent plant viral pathogens, infecting over one thousand (1,000) plant species (Ng and Perry, 2004; Akhtar *et al.,* 2010). Recently, aphids were found to be associated with a novel necrotic disease on cabbage in Ghana, characterized by mosaic, necrosis, stunting and subsequently death of plants (Fening *et al*., 2016; Forchibe, 2016, Adenka *et al*., 2021). The disease is suspected to be of viral aetiology, and has been reported to cause complete yield loss once reported on cabbage fields (Adenka *et al*., 2021). Thus, it is important to identity the disease causal pathogen as well as understand the role of aphids in the transmission of the novel necrotic disease, as it is unsure if aphids are the authentic vectors or if the presence of the disease on cabbage predisposes them to attack by aphids.

There was therefore the need to study the diversity of aphids on cabbage in Ghana, important aspects of their bioecology and understand their association with the novel necrotic disease, as a prerequisite to developing quarantine measures and sustainable management interventions.

1.3 Objectives

The main objective of this study was to determine the diversity of aphids present on cabbage in Ghana and understand some important aspects of their bioecology in association with a novel necrotic disease on cabbage.

The specific objectives of the study were to:

I. ascertain the species of aphids present on cabbage, their distribution, and density in five agroecological zones of Ghana.
II. determine the spatial density patterns and model the habitat suitability of identified aphids.
III. determine the incidence and seasonal variation of aphids and their associated natural enemies on cabbage.

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IV. study the bionomics of the two dominant aphids on three cabbage varieties commonly grown in Ghana.

V. identify and characterise the disease-causing pathogen associated with aphids' infestation on cabbage

VI. assess farmers' perception of aphids as pests of cabbage in Southern Ghana.

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CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Origin and distribution of cabbage

Cabbage, *Brassica oleracea* var. capitata L. (Family: Brassicaceae), is an important leafy vegetable derived from the wild type, *Brassica oleracea* L. (also known as colewort) (Nieuwhof, 1969). Other varieties of the brassica family include cauliflower, broccoli, kale, and brussels sprout. Cabbage is indigenous to Southern Europe and the Mediterranean regions, but is now cultivated throughout the world (FAO, 2000; Bhatia *et al.*, 2011). It is considered as one of the oldest vegetables and was mentioned in early literature to be in use as early as 2000 or 2500BC by ancient Romans and Greeks (Chiang *et al.*, 1993; Economic Research Service (ESR), 2002). Different types and varieties of cabbage show considerable variation with respect to the size, shape, color, and texture of the head (Singh *et al.*, 2006). Earlier classifications by Nieuwhof (1969), categorized cultivated cabbage into red cabbage, savoy cabbage, and white cabbage.

It is unclear when the cultivation of cabbage commenced in Ghana (Cobblah *et al.*, 2012) but is believed to have been introduced with the incursion of the British on the Gold Coast in the 1940s (Sinnadurai, 1992). Since then, there has been a growing popularity of cabbage in Ghana especially in urban areas, and several varieties have been developed to suit consumers' tastes and resist environmental stress. Due to its high nutritional content, cabbage has now become a major part of peoples' diet in the country. Timbilla and Nyarko (2004) recorded over nine varieties of cabbage cultivated throughout the country (Capitata, Copenhagen market, Oxylus, KK cross, Holland, Tenzier, KY cross, Gloria, and Master globe) but in recent times, newer varieties such as Leader

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cross, Santa, Fortune, Tropica cross, Super cross, Tropica king, Super comet, and Milor (Agriseed Ltd, Ghana) have been developed to suit the changing climatic conditions.

2.2 Nutritional attributes and economic importance of cabbage

The top ten major producers of cabbage include China, India, Russia, Japan, South Korea, Ukraine, Indonesia, Poland, Romanian, and the USA, with China being the largest; with about 50% of the world's production (FAO stats, 2011; World atlas, 2017). Cabbage is considered an important crop with a huge demand from the culinary industry across the globe. It is rich in nutrients such as carbohydrates, vitamins, alkaloids, tannins, minerals, and glutamine (Oguwike *et al.*, 2014; Parveen *et al.*, 2016). Its richness in phytochemical components makes it have anti-inflammatory, antioxidant, and anticarcinogenic effects (Tajalli *et al*., 2020).

In West Africa, cabbage is mostly grown by small-scale farmers for the local and the surplus for the international markets (Kouamé *et al.*, 2006; Probst *et al.*, 2012; Owusu-Boateng and Amuzu, 2013). It is mostly cultivated in peri-urban areas and supplied into major cities. In Ghana, Timbilla and Nyarko (2004) reported that cabbage is grown in all regions of the country except the Upper East and Western regions with up to 49% of farmers growing all year round. Other studies have indicated an increase in the cultivation of cabbage across the country, attributed to an increase in demand and the population of foreigners in some urban cities (Osei *et al*., 2013). The cultivation of cabbage in Ghana provides livelihood for a large number of small-scale farmers, thus, contributing to food security and income generation leading to poverty reduction (Timbilla and Nyarko, 2004; Mochiah *et al.*, 2011).

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2.3 Constraints to the production of cabbage

Cabbage, though important with enormous benefits for consumption, faces severe biotic and abiotic constraints during production. Of the biotic constraints, are insect pests whose attack causes a significant reduction in yield and quality of cabbage, often leading to frequent pesticide applications (Zehnder *et al.,* 1997; Mpumi *et al.,* 2020). Pesticides have been reported to have adverse effects on the environment, health and non-target organisms, thus, purported as an issue of great concern (Obeng-Ofori, 2007).Major pests that attack cabbage include; Diamondback moth (*Plutella xylostella*), Aphids (*Brevicoryne brassicae, Lipaphis pseudobrassicae, Myzus persicae*), cabbage webworm (*Hellula undalis*), Flea beetles (*Phyllotreta* sp.), Cabbage looper (*Trichoplusia ni*), cabbage white butterfly (*Pieris rapae*), Whitefly (*Bemisia tabaci*) (Shelton *et al*., 1988; Amoabeng *et al*., 2013; Fening *et al*., 2014). Of this complex of arthropod pests attacking cabbage are aphids whose ability to vector plant viruses makes them one of the most economically important groups of pests in horticulture or agriculture in many parts of the world (Dixon, 1985; *G*uerrieri and Digilio, 2008).

Abiotic constraints identified with cabbage production includes; availability of land, irregular water supply, weed competition, lack of credit facilities, high cost of inputs, and marketing of crops (Bangnikon and Ofori, 1996; Timbilla and Nyarko, 2004; Osei *et al*., 2013).

2.4 Aphids as pests of crops

Aphids are among the major insect pest groups, globally causing significant economic damage to many food and commodity crops. Those of agricultural importance are known to be mostly from the subfamily Aphidinae, with more than 4000 species identified and over 250 species noted as

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crop pests (Blackman and Eastop, 2007). Aphids damage crops in several ways: firstly, as phloem-feeders, they suck plant sap which leads to stunting and sometimes death of plants in heavy infestations. Secondly, aphids excrete 'honeydew' which is suitable for the growth of a black sooty mold which reduces the cosmetic appearance of crops and photosynthetic activity (Hughes, 1963). Thirdly, they also inject saliva into plants during feeding which could be phytotoxic (Dedryver *et al.*, 2010). Lastly, aphids are major vectors of plant viruses, transmitting over half of insect-vectored viruses (Nault, 1997). Their enhanced ability to reproduce rapidly, for example; one mature aphid can give birth to 2 to 5 live young per day which matures between 5 to 7 days, further raises their pest status (Myburgh, 1993).

Aphids have complex and varied life cycles. Major taxa exhibit two major life cycles which include holocyclic (periods of very low temperatures and reduced photoperiods; where males are produced) and anholocyclic (during warm periods, by parthenogenesis) (Fig 2.1) (Kessing and Mau 1991; Blackman and Eastop, 2000; Agarwala, 2007). Holocyly is characterized by the presence of an egg stage which is usually deposited before winter. It then hatches into a fundatrix (a wingless female aphid) which subsequently produces daughter clones (Flint, 2000). These females then produce both males and females which mate, and the females lay eggs that enter diapause to survive the winter. In anholocycly, female aphids reproduce parthenogenetically, giving birth to live clones of themselves that are already carrying a developing aphid embryo (Flint, 2000). Nevertheless, cosmopolitan species follow both holocyclic and anholocyclic life cycles, concurrently in different geographical locations (Bhatia *et al.*, 2011).

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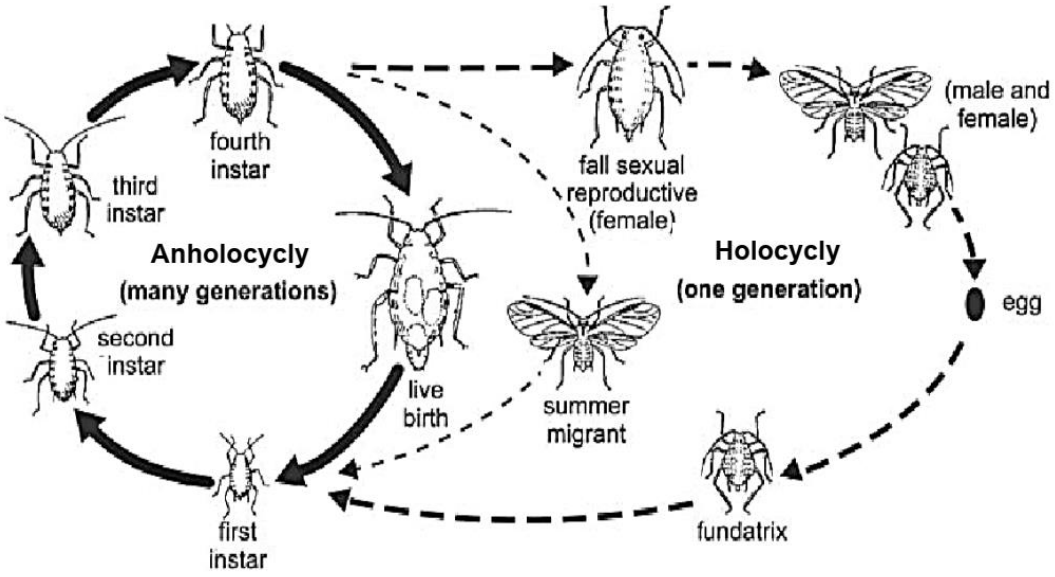


Figure 2-1: Generalised life cycle of aphids (Source: Flint, 2000).

2.4.1 Major features of aphid identification

Aphids can be primarily recognized by several shared morphological characteristics such as siphunculi, five or six-segmented antennae, two-segmented tarsi, and cauda often used for thrusting off honeydew from the anus (Blackman and Eastop, 1984; Van Emden and Harrington, 2007). However, these features may vary depending on environmental and ecological factors (Saguez *et al.*, 2013). The waxy secretion of some aphids is important for identification (Blackman and Eastop, 1984). However, inadequate distinguishing morphological traits or high levels of polymorphism exhibited by aphids makes it difficult for them to be morphologically differentiated (Blackman and Eastop, 1984). For this reason, further suggestions were made by Blackman and Eastop (2000) that non-physically confusing features be used for morphological delimitation among aphids. Furthermore, recent advances in molecular methods and tools for species identification would be the best option for ascertaining the identity of a particular species.

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2.5 Aphids that occur on cabbage

Thirteen species of aphids have been recorded as feeding on *Brassica oleraceae* worldwide, Blackman and Eastop (2000), which include: *Aphis craccivora, fabae, gossypii, nasturtii, spiraecola; Aulacorthum prasinum, solani; Brachycaudus helichrysi; Brevicoryne brassicae; Dyaphis montemartinii ghanii; Hyalopterus pruni; Lipaphis pseudobrassicae (erysimi); Macrosiphum euphorbiae; Myzus antirrhinii, ascalonicus, ornatus, persicae; Pemphigus populitransversus*; *Protaphis middletonii*; *Rhopalosiphoninus latysiphon*; *Sitobion miscanthi and Smynthurodes betae.* Of these, the green peach aphid, *Myzus persicae* (Sulzer), the Indian mustard aphid; *Lipaphis* erysimi *pseudobrassicae* (Davis), and cabbage aphid, *Brevicoryne brassicae* (Linnaeus) are of economic importance in cabbage cultivation. Nevertheless, as with other pest species, their distribution, abundance and economic status vary globally depending on climatic factors and food availability (Bebber *et al*., 2014).

The polyphagous aphid, *M. persicae* has successfully colonized hundreds of plant species belonging to over 40 families (Flanders *et al.,* 1992; Blackman and Eastop 2000; CABI, 2015). *Lipaphis* e. *pseudobrassicae* and *B. brassicae* are known to feed specifically on cruciferous crops, and attack cabbage from the nursery till harvest, while *M. persicae* attacks cabbage before heading begins (Elwakil and Mossler, 2013). In the tropics, *L. e. pseudobrassicae* is noted as the most damaging aphid on brassica and is often referred to as the false cabbage aphid due to its close resemblance to *B. brassicae* (Ronquist and Ahman, 1990) which is also noted in the temperate and subtropical regions as the most damaging aphid on brassica (Blackman and Eastop, 2007).

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2.5.1 The mustard aphid; *Lipaphis erysimi pseudobrassicae* (Davis, 1914)

2.5.1.1 Taxonomy and distribution

Lipaphis erysimi pseudobrassicae (Hemiptera: Aphididae) (=*Lipapahis erysimi* or *Lipaphis pseudobrassicae)* is a major brassica pest, native to Asia where it has an extensive distribution (Bakhetia and Sohi, 1980), but has over the years been introduced into other countries around the world. This insect was first confused for *B. brassicae* in North America until Davis (1914) identified it as a different species (Essig, 1948). The Lipaphis group constitutes mainly of two morphologically similar but geographically distinct subspecies; the European form (*Lipaphis erysimi erysimi)* and the Asian form (*Lipaphis* erysimi *pseudobrassicae)* (Müller, 1986; Ronquist and Ahman 1990). The European forms only occur on wild crucifers, are holocyclic, and not usually associated with brassica crops (Müller 1986). Between the 1970s-2000s, both sub-species were referred to as *Lipaphis erysimi* due to difficulty in distinguishing them (Müller, 1986; Liu *et al.*, 1997; Blackman and Eastop, 2000). Nonetheless, some studies still refer to *Lipaphis erysimi* as the widely distributed pest of brassica (Srivastava and Gularia, 2003; Rana, 2005; James *et al.*, 2010, Saethre *et al.*, 2011; Adhab and Schoelz 2015; Patel *et al.*, 2017a).

L e. pseudobrassicae is distributed in both the tropical and temperate regions of the world feeding mainly on brassica crops. The apterae are yellowish, grey, or olive green with a waxy layer which becomes denser in humid conditions. They have a body length of 1.5-2.3 mm and occur in large colonies under cabbage leaves, leaving the leaves wrinkled. 'Winged females have dusky green abdomens with dark lateral stripes' (Blackman and Eastop, 1984). The antennae are dark, and pale at the base (Blackman and Eastop, 1984). The major distinguishing features from other aphids are

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the non-convergence of the frontal tubercles; the pale cornicles which are longer than the cauda; the tongue-shaped cauda and a thin layer of white waxy secretion on the colonies (Blackman and Eastop, 1984; Adhab and Schoelz, 2015).

2.5.1.2 Life cycle of *L. e pseudobrassicae*

Lipaphis erysimi pseudobrassicae undergoes anholocyclic reproduction in most regions of the world (Blackman and Eastop, 2000) and males are very rare. However, holocycly has been recorded on cabbage in Western Honshu, Japan (Kawada and Murai, 1979). Without much evidence of the occurrence of oviparous females, only ovoviviparous forms have been reported to occur on crops throughout the year even in both warm and cold climates. The mustard aphid is highly prolific and can have up to 11–25 and 35 generations annually in temperate and tropical conditions respectively (Capinera, 2008).

2.5.2 The cabbage aphid; *Brevicoryne brassicae* (Linnaeus, 1758)

2.5.2.1 Taxonomy and distribution

Brevicorryne brassicae (Hemiptera: Aphididae) has a body length of 1.6 to 2.8 mm, covered with a greyish waxy covering, with thick and very short siphunculi and broad triangular cauda (Blackman and Eastop 1984; Carter and Sorensen 2013). The short cornicles and thick greyish waxy secretion are the major distinguishing features from the other aphids (Blackman and Eastop 1984; Opfer and McGrath, 2013). A concise account of the biology and pest status of the cabbage aphid has been provided by Gabrys *et al*. (2008) in the encyclopedia of entomology. It is known

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to be native to Europe and is distributed throughout the temperate and warm temperate regions of the world (Blackman and Eastop, 2000).

2.5.2.2 Life cycle of *B. brassicae*

Reproduction in *B. brassicae* is anholocyclic during warm periods, (Kessing and Mau 1991) and holocyclic during lower temperatures; during which there is the production of males (Blackman and Eastop, 1984). After mating occurs, the females lay eggs that overwinter, and nymphal development takes 7-10 days and differs from the adults in having less developed cauda and siphunculi. Several generations occur within the life of one aphid, with over 15 generations during one cropping season (Hafez, 1961; Hines and Hutchison 2013).

2.5.3 The green peach aphid; *Myzus persicae* (Sulzer, 1776)

2.5.3.1 Taxonomy and distribution

Myzus persicae (Hemiptera: Aphididae) is an extremely polyphagous and highly polymorphic aphid, with colours varying from pale greenish-yellow to pink, red, or almost black and has an average body length of 1.2-2.3 mm (Blackman and Eastop, 1984; Van Emden and Harrington, 2007). The distinguishing features from other brassica aphid species include prominent and inward-pointing antennal tubercles, and the base of the cornicles are swollen, and they are longer than the cauda (Blackman and Eastop, 1984). Winged forms have a shiny black fleck on the rear part of the abdomen. It is presumed to originate from Asia, where its primary host, *Prunus persica* (L.) Batsch, is native, and is now distributed worldwide on diverse plant species (Jansson, 2003; Van Emden and Harrington, 2007).

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2.5.3.2 Life cycle of *M. persicae*

Myzus persicae is heteroecious holocyclic (host alternating, with sexual reproduction during part of its life cycle) between *Prunus* (usually peach) and summer host plants, but anholocyclic on secondary (summer) hosts in many parts of the world with favorable environmental conditions (Broadbent, 1949; Blackman, 1974; Blackman and Eastop 2000). It undergoes anholocycly in the tropics and sub-tropics on a wide variety of plants, including many vegetable crops such as cabbage and its *Brassica* relatives, potato and other crops of the family Solanaceae and celery, mustard, pepper, pumpkin, okra, corn, sunflower, and other flower crops (Blackman, 1974; Margaritopoulos *et al.,* 2002). An aphid starts producing about 12days after birth and there can be 20 generations in a year during warmer climates (Blackman, 1972).

2.6 Variations in aphid populations on brassica due to biotic and abiotic factors

Colonies of aphids on cabbage consist mainly of apterous females and nymphs during the major growing seasons and several alate forms in unfavorable conditions. Their abundance on crops fluctuates based on host plant quality, inter-specific and intra-specific abundance, host plant availability and density, and environmental factors (Van Emden and Bashford, 1971; Dixon, 1977; Agarwala and Datta, 1999) which can be termed biotic and abiotic factors respectively. Therefore, different seasons affect physiological processes, population build-up, distribution, and colonization of new habitats. They usually exhibit an exponential growth rate when favourable environmental factors are fairly constant (Honěk, 1987). However, as overcrowding becomes evident their rate of increase decreases due to phenological alterations of their host plants as well as other climatic factors or they may migrate (Moran, 1992). Amongst the abiotic factors that affect

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insect abundance and distribution, temperature and relative humidity stand out to be the most constraining factors (Savopoulou-Soultani *et al.,* 2012). Studies have shown the influence of abiotic factors on aphid population on cruciferous crops (Fidelis *et al*., 2018; Soh *et al*., 2018; Shonga and Getu, 2020). Biotic factors that affect aphid populations include plant phenology, natural enemies such as predators, parasitoids, and naturally occurring entomopathogens. Associated natural enemies of aphids are discussed in Section 2.8.6 of this review.

2.7 Economic importance of aphids on cabbage

The economic importance of aphid species is dependent on the type and extent of damage caused, and on the economic importance of the hosts (Dedryver *et al.*, 2010). Losses due to aphid infestation on brassica crops have been estimated at approximately 35 – 91 % under different environmental conditions (Singh and Sachan, 1994). The intensity of this damage is often influenced by the plant growth stage, the duration of infestation, plant vigour, and environmental conditions. Munthali *et al*. (2004) recorded rapid population build-up on cabbage in Botswana during warm weather, accompanied by severe damages to the crops. Rana (2005) reported 10-90% damage caused by *Lipaphis erysimi* depending on the severity of infestation and plant stage while in India, Singh and Sharma (2002) reported damage ranging from 9-96% in different agroecological zones. Losses due to *B. brassicae* and *L. erysimi* were estimated at 70-80% on oilseed brassica in Pakistan (Razaq *et al.,* 2011). Other brassicas like *Brassica napus, Brassica juncea*, and *Brassica carinata* had severe losses from aphid infestation of up to 75.06%, 77.25%, and 81.86% respectively (Razaq *et al.,* 2011). *Brevicoryne brassicae* has been reported to cause total crop loss in leafy brassica crops in Ethiopia (Munthali and Tshegofatso, 2014). In Senegal and the Democratic Republic of Congo, major aphids occurring on cabbage are often *M. persica*,

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B. brassicae, and *L. erysimi* which causes rolling of young leaves, chlorosis, deformation, and thickening of leaves in severe outbreaks (Yarou *et al.*, 2017). Yarou *et al*. (2017) further reported that the most important damage by these pests on cabbage is the transmission of viruses and sooty mold formation following honeydew production. Mersha *et al*. (2014) reported that aphids reduce the yield of cabbage and its aesthetic and nutritional content. In Ghana, the two major aphid species reported to be causing huge losses (20-100%) and damage in major cabbage-producing regions are *M. persicae*, and *L. pseudobrassicae* (Forchibe *et al*., 2017). Adenka *et al.* (2021), reported 100% loss to aphids in six different cabbage varieties in Ghana. Some reports have concluded that aphid infestation often reduces plant growth and in cabbage, it usually leads to the death of plants (Munthali and Tshegefatso, 2014; Embaby and Lotfy, 2015).

2.7.1 Aphids as vectors of plant viruses

Transmission of viruses by insects is one of the major problems associated with the intensification of vegetable and fruit production often leading to huge losses due to quality, with several unmarketable products (Moreno *et al.*, 2004; Dedryver *et al.*, 2010). Tomlinson (1987) reported that the major economically important viruses transmitted by aphids in field-grown vegetables, includes the *Cucumber mosaic virus* (CMV), *Lettuce mosaic virus* (LMV), *Papaya ringspot virus* (PRSV), *Turnip mosaic virus* (TuMV), and *Potato virus Y* (PVY). The efficiency of virus spread by aphids is linked to their capacity to make intracellular probes on leaves without killing the cell (Wamonje *et al*., 2020). When favorable content is found, aphids probe deeper into sieve elements, and in the process, acquire virus particles (Tjallingii and Esch, 1993). Probing on the epidermal cells of leaves often suffices for the acquisition of non-persistently transmitted viruses while phloem-feeding is usually correlated with the acquisition of persistently transmitted viruses (Brault

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et al., 2010). Virus transmission by aphids involves acquisition, retention, inoculation, and association with the vectors (Brault *et al.,* 2010). The major viruses infecting cabbage includes *Cauliflower mosaic virus* (CaMV), *Turnip mosaic virus* (TuMV), *Beet western yellows virus* (BWYV), and sometimes *Cucumber mosaic virus* (CMV), all of which are vectored and transmitted by aphids (Moreno *et al.*, 2004; Sevik and Deligoz, 2017). CMV (Moreno *et al.*, 2004) and TuMV (Nguyen *et al.*, 2013) are noted as the most widespread and damaging pathogens of brassicas worldwide, followed by the CaMV (Spence *et al.*, 2007). The former is transmitted by *M. persica, L. erysimi* and *B. brassicae* non-persistently (Day and Venables, 1961), and BWYV is persistently transmitted by *M. persicae* (Kyriakou *et al*., 1983).

Brevicoryne brassicae is known to vector and transmit over 23 viral pathogens in a non-persistent mode (Eastop, 1977). *Myzus persicae* transmits over 100 plant viruses in many economically important crops (CABI Plantwise, 2020), and is considered among the most successful aphid vectors of plant viruses due to its polyphagous nature (Ng and Perry, 2004). *Lipaphis e. pseudobrassicae* is reported to vector and transmit at least 16 plant viruses (Blackman and Eastop, 1984). Ghosh *et al*. (2017) reviewed aphids as vectors of plant viruses.

2.8 Management of aphids on cabbage

Aphid management on cabbage had earlier been geared towards the use of insecticides because of their accessibility, availability, and rapid action (Dewar *et al.*, 2017). In Ghana, insecticides remain the main control option for aphids on many crops, however, there has been a push for more environmentally friendly practices as a segment of an Integrated Pest Management (IPM) strategy (Ntow *et al*., 2006; Horna *et al.*, 2008; Forchibe *et al*., 2017). IPM aims to augment beneficial

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insect populations, avert pest outbreaks, pest resurgence, and disease spread, while maintaining the natural environment (Mangan and Mangan, 1998; Afreh-Nuamah, 2003; Naranjo *et al*., 2015). IPM combines different pest management strategies with judicious pesticide use, usually as the last resort, to achieve production goals. Multiple IPM approaches have been suggested for the management of aphids on brassica crops (Farooq and Tasawar, 2008; James *et al*., 2010; Liu *et al*., 2014; Reddy, 2017).

2.8.1 Mechanical control

Mechanical control measures are usually recommended to be considered first when pest populations have not exceeded threshold limits. They often include a wide range of practices such as the use of traps, hand crushing, erecting barriers, removing infested plants or leaves, and conducting activities that disrupt pests feeding and multiplication (Pesticide Research Institute). For example, Griffin and Williamson (2012) suggested that fields be furrowed immediately after harvest to prevent aphids spreading to other crops. Clearing alternative host plants like mustard and other cruciferous weeds around fields also reduces the probability of infestation (Natwick, 2009). Other reports have shown that trap crops also prevent aphid infestation of the main crop. Crops like dill, nasturtiums, and timothy grass planted close to the main crop have been reported to deter aphids (Philbrick and Gregg, 2012). Aphids can also be washed off plants using a high-pressure water hose with jet-set dial nozzles. The Landscape IPM showed that using jet or shower setting nozzles easily dislodges insects from plants. Although mechanical control measures as important, they are only practical in small gardens and cannot be adopted in large-scale cultivation.

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2.8.2 Monitoring and sampling

Monitoring of aphids is a useful tool, aimed at providing information for timely and efficient implementation of management strategies. Several methods are used in aphid monitoring depending on the purpose. As vectors of plant viruses, there is almost zero tolerance for aphids on brassica crops, hence the need for monitoring. Harrington *et al*. (2007) provided a review of techniques in aphid monitoring. In their review, sampling methods were grouped into direct which includes: in situ plant counts, destructive plant counts, vacuum sampling, and indirect which includes the use of coloured water pan traps, clear or plant-coloured water pan traps, sticky traps, filter nets, and suction traps. Direct sampling methods are often used in cabbage production because they provide a measure of absolute abundance per plant, which is usually requisite for population density estimates. Monitoring provides threshold data for the implementation of pest management interventions. In USA and UK, treatment thresholds have been established for *B. brassicae* (Ellis *et al*., 1999; Collier and Finch, 2007) and *L. pseudobrassicae* in brassica crops in Asia (Singh and Malik, 1998). Pest management guidelines for the USA suggested that cabbage crops should be treated as soon as 1-2% of plants were infested with *B. brassicae* since it has a low action threshold (Collier and Finch, 2007). However, infestations of up to 100 aphids/plant for *M. persicae* could be tolerated (Eastman *et al*., 2005). In Kenya, Pests Management Decision Guide suggests the institution of control measures when 1 to 2% of plants are infested with either of the three major aphids (Murage *et al.*, 2014). Despite the availability of this information in some countries, and it is incumbent on the growers to utilize them, nevertheless, information on how widely adopted and used these thresholds are have not been determined (Collier and Finch, 2007). However, such action thresholds are very useful for the implementation of IPM approaches.

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2.8.3 Cultural control

Cultural control of aphids involves the reduction of aphid damage through manipulations of the physical and biological environment of the crop before and during growth (Wratten *et al*., 2007). Some methods are aimed at improving plant vigour, crop yield, etc, which may not directly have impacts on the aphids (Perring *et al*., 2018). Farm sanitation for example is very effective for the improvement of plant growth and at the same time kills over overwintering aphid eggs in temperate climates and prevents farm re-infestation (Hines and Hutchison, 2013). Sowing dates have been shown to have a significant effect on aphid populations while the crops sown late in the season suffer aphid infestation (Sidiqui *et al*., 2009; Dharavath *et al*., 2016).

Intercropping is a cultural approach employed by several growers to manage aphids in the production of brassica crops. This approach is such that non-host plants offer resources to natural enemies, thus augmenting their populations, and interfere with the host finding ability of specialist herbivores such as aphids (Root, 1973). Subsequently, several authors have reported on the effects of intercropping in controlling pests of cabbage (Ponti *et al*., 2007; Mochiah *et al*., 2011; Baidoo *et al*., 2012). Ben-Issa *et al*. (2017) reviewed companion plants used in aphid pest management in the categories of trap plants, alteration of host plant selection, and natural enemy attractants.

2.8.4 Host plant resistance

Plant resistance to insects is often a result of heritable characters that result in less damage compared to plants without these characters. Stout (2014) in his book, explores and discusses host plant resistance (HPR) approaches and integration into IPM strategies, outlining the major steps involved in development and implementation. In a review by Van Emden (2007), 12 mechanisms

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of resistance to aphids were discussed and classified under the three major classes: antixenosis, antibiosis, and tolerance. Breeding for plant resistance against insects is not common as is the case of breeding disease-resistant cultivars partly due to the availability of a wide range of rapid control measures. Sharma and Ortiz (2002) attributed this scarcity to the difficulty involved in screening insect-resistant plant varieties, the high cost of rearing programmes, and long years of technology development which may not yield the expected results. Bhatia *et al*. (2011) suggested that breeding for genetic resistance against aphids is difficult, due to the absence of resistant sources within the crossable germplasms and insufficient knowledge in the associated genetics. Several studies have been carried out with cabbage lines to screen for resistant varieties to *B. brassicae, L. pseudobrassicae* and *M. persicae* (Ahmed *et al*., 2018; Taghizadeh, 2019; Hong *et al*., 2019; Ali *et al*., 2021). HPR can be used an important strategy for the integrated management of aphids on cabbage.

2.8.5 Chemical control

The use of chemicals in the management of crop pests dates back to the times of the organochlorines, organophosphates, carbamates, and recently pyrethroids and neonicotinoids (Ware and Whitacre, 2004; Davies *et al*., 2007). With frequent food safety concerns with the use of synthetic insecticides, alternative options have been recommended for pest control (Fening *et al*., 2013, 2014; Forchibe *et al*., 2017). Tolerance to aphids as a pest is minimal, owing to their rapid reproduction and resistance to insecticides (Ahmad and Akhtar, 2013; Bass *et al*., 2014). In this light, several studies have been done to develop new compounds for their management (Tang *et al*., 2019; Singh and Joshi, 2020). Dewar and Denholm (2007) reviewed the chemical control of aphids stating the progressive shift from more persistent pesticides to less persistent and

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environmentally friendlier options. With the increasing demand for cabbage, most farmers have relied on the use of insecticides as it gives fast results (Ntow *et al*., 2006; Owusu-Boateng & Amuzu, 2013; Machekano *et al*., 2019). However, with the increasing concern on the negative impacts of these chemicals on the environment and non-target organisms, there is a gradual shift to safer options. Development of insecticides moved from broad-spectrum persistent materials to short-lived, effective, sometimes expensive, and specific materials which are environmentally friendly (Perring *et al*., 2018).

Several authors have evaluated the efficacy of different insecticides against aphids on brassicaceous crops; effects of imidacloprid (Neonicotinoid) on aphids on cabbage (Djomaha *et al*., 2016), effects of flupyradifurone (Butenolide) on the green peach aphid, *M. persicae* (Tang *et al*., 2019), effects of acetamiprid (Neonicotinoid) on *L. pseudobrassicae* (Maia *et al*., 2020), effects of cyantraniliprole (Diamide) and imidacloprid on *B. brassicae, M. persicae* (Ahumada and Chorbadjian, 2019), among others. With the growing demand for organic vegetables, less toxic options for pest management have been suggested to meet up with the growing market. Much research work has been done on the effects of botanicals on the population of aphids on cabbage: Baidoo and Adam (2012) recorded larger yield in cabbage when neem seeds and *Lanthana camara* leaf extracts were used to control pest, Fening *et al*. (2014) observed insecticidal effects of garlic and hot pepper extracts against insect pests on cabbage with minimal effects on natural enemies, Djomaha *et al*. (2016) and Forchibe *et al*. (2017) recorded positive control of aphids on cabbage using neem seed extract, Pissinati and Ventura (2015) suggested neem oil and kaolin oil as alternatives for the control of cabbage aphids on kale. Pereira *et al*. (2019) reported insecticidal properties in *Agave americana* against aphids on cabbage. He further recommended its extract as a good alternative to synthetic insecticide against aphids on cabbage. A study by Jabran *et al*.

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(2016), reported control of the cabbage aphid by 50% through allelopathic water extracts of sorghum, sunflower, brassica and mulberry. Great success has been recorded for many botanicals against insect pests and some; especially neem pesticides are now commercially produced and exported for use in vegetable production. The safety and efficacy of these neem-based pesticides have been tested and recommended for use by several researchers (Obeng-Ofori, 2008; Lokanadhan *et al.*, 2012).

Insect growth regulators (IGRs) are also a group of chemical compounds that alter growth and development in insects; as juvenile hormones, as precocenes, and as chitin synthesis inhibitors (Ishaaya, 2001). Juvenile hormones (JH) include ecdysone (the moulting hormone), JH mimic, JH analog (JHA), and are known by their broader synonyms, juvenoids and juvegens. They disrupt immature development and emergence as adults. Precocenes interfere with the normal function of glands that produce juvenile hormones. Chitin synthesis inhibitors, (conventional benzoylureas, buprofezin and cyromazine), affect the ability of insects to produce new exoskeletons when molting (Horowitz and Ishaaya, 2004). Neem products have been reported to act as IGRs and are very useful in controlling insect pests (Lokanadhan *et al.*, 2012). Though IGRs seem to be very effective in their mode of action, they are non-specific and often affect beneficial arthropods. Cabral *et al.* (2008) reported that buprofezin (a chitin synthesis inhibitor) affected the survival, fecundity, fertility and egg hatching of the major aphid predator *Coccinella undecimpunctata.*

2.8.6 Biological control

Biological control is the use of natural enemies; predators, parasitoids and pathogens to control insect pests. The natural enemy populations of aphids are often numerous enough to keep their

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population below economic damage thresholds (Rabasse and van Steenis, 1999; Mandal and Patnaik, 2008). Sometimes, wasp parasites are the most effective; in other cases, predators, such as the hoverflies, lady beetles, or entomopathogens such as fungi cause the most mortality (Liu *et al.,* 2014; Fidelis *et al.,* 2018). Although there is a rich diversity of natural enemies of aphids, biological control is often not efficient (Dixon, 2000; Sampaio *et al.,* 2017). Perring *et al*. (2018) explained that this unsatisfactory result gotten from biological control can be attributed to the incompetence of aphid natural enemies as agents of biological control. Pal and Singh (2012) rather suggested that, under natural conditions, 'before parasitoid populations are large enough to be effective, the aphid population has usually exceeded the Economic Injury Level (EIL).' Other factors include hyperparasitism of main aphid parasitoids, the complexity of implementation of a biological control programme as it takes into consideration all factors that affect the pest unlike using insecticides. Thus, implementation involves high levels of skilled personnel.

2.8.6.1 Natural enemies of aphids on brassicas

The natural enemies of aphids in brassicas range from entomopathogenic fungi such as those of the genus *Lecanicillium,* (Vu *et al.,* 2007) and Entomophthorales (Scorsetti *et al.,* 2007) to Braconid and Aphelinid parasitoids (Geiger *et al.,* 2005; Sampaio *et al.,* 2017) and predators, of the families Coccinellidae, Syrphidae and Chrysopidae (Debaraj & Singh, 1998; Nieto *et al.,* 2006). Additionally, some Aranae are good predators of cabbage pests (Amoabeng *et al.,* 2013; Grez *et al.,* 2014; Forchibe *et al.,* 2017). They hunt or use webbing to catch their prey, and experiments have demonstrated their ability to control aphids (Madsen *et al.,* 2004; Dippenaar-Schoeman *et al.,* 2013). Liu *et al*. (2014) discussed the diversity of natural enemies associated with

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brassica vegetables in China, detailing the bioecology and some ecological aspects of major natural enemies in the region.

Sæthre *et al*. (2011) observed the following predators to be associated with *L. erysimi* and *M. persicae* on cabbage and turnip in Benin; *Cheilomenes propinqua, Hippodamia varigata, Ischiodon aegyptius,* and *Cheilomenes sulphurea*. Fidelis *et al*. (2018) reported the following natural enemies associated with *B. brassicae* on cabbage; *Allograpta exotica*, *Pseudodorus clavatus*, *Ocyptamus gastrostactus* (Syrphidae larvae) *Coccinella septempunctata*, *Cycloneda sanguinea*, *Eriopis connexa* and *Harmonia axyridis (*Coccinellidae). He concluded that predation is the major mortality factor of *B. brassicae* in cabbage crops. Of the huge diversity of predators associated with aphids on cabbage reported around the world *C. septempunctata* has been reported to be the most efficient predator of *L. erysimi* under field conditions (Shenhmar and Brar, 1995; Singh and Singh 2013).

The cosmopolitan parasitoid *Diaeretiella rapae* (M'Intosh) (Hymenoptera: Braconidae), is the major natural enemy of *B. brassicae, L. pseudobrassicae, M. persicae,* and aphids of other crops (Zhang and Hassan, 2003; Agbogba, and Powell, 2007; Khakasa *et al.*, 2016). However, the inefficiency of this parasitoid has been attributed to the very low parasitism levels observed by Lopez and Van Driesche (1989) and Zaki *et al.* (1999). Sampaio *et al*. (2017) also reported high hyperparasitism of *D. rapae* by *Alloxysta fuscicornis,* which often has the same distribution on fields as the host. Sæthre *et al*. (2011) reported *Lysiphlebus testceipes* association with *L. erysimi* on cabbage in Benin. Sawar (2014) reported *Aphidius* sp. as the most abundant parasitoid parasitising *M. persicae* on *Brassica napus*. *Aphelinus* sp, was reported to parasitise *B. brassicae* and *M. persicae* in United Kingdom (Blande *et al.*, 2008; Van Emden *et al.*, 2008).

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Entomopathogens play a very vital role in the management of pests and have been used extensively for the control of vegetable pests. *Aspegillus versicolor*, *Verticillium lecanii*, *Beauveria bassiana* have been assessed for use on *B. brassicae* and *M. persicae* with a positive outcome (Yeo *et al.*, 2003; Asi *et al*., 2009; Embaby and Lofty, 2016; Pacheco *et al.*, 2017). *Lecanicillium lecanii*, *Paecilomyces lilacinus*, *B. bassiana* and *Metarhizium anisopliae* were reported to be efficacious against *L. erysimi* (Ujjan and Shahzad, 2012). Most of them are commercially available in multiple formulations. Some have been shown to be better alternatives to chemical insecticides. Maina *et al*. (2018) outlines the use of entomopathogenic fungi for biocontrol of insect pests, concluding that, there is need for further research to discover more fungi which are pathogenic to insects.

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CHAPTER 3

3.0 Diversity, distribution, and identification of aphids on cabbage in Ghana

3.1 INTRODUCTION

Cabbage, *Brassica oleracea* var capitata is a biennial vegetable grown in Ghana from the North to the South annually mostly in urban and peri-urban areas by small-holder farmers. It is grown across a wide range of environmental conditions but is highly suited for cooler climates. It is attacked by a multitude of pests, with aphids being one of the most destructive, attacking plants from nursery to maturity (Forchibe *et al*., 2017). Aphids have recently been noted as key pests of cabbage in Ghana especially due to their association with a novel mosaic necrotic disease observed on cabbage, which usually leads to 100% loss (Fening *et al*., 2016; Adenka *et al*., 2021). All stages of aphids suck plant sap, leading to necrosis, decrease in plant nutrients, loss of plant vigour, stunting, and subsequently death of plants. *Brevicoryne brassicae, L. e. pseudobrassicae* and *M. persicae* have been reported to be the major aphid species associated with cabbage worldwide, their establishment and distribution are often dependent on climatic factors, land use and availability of host plants (Cocu *et al*., 2005). *B. brassicae* and *L. e. pseudobrassicae* feed specifically on brassicaceous crops, while *M. persicae* feeds on a wide variety of crops. Significant damage on crucifers associated with *L. e. pseudobrassicae* has been reported in African countries including Benin, Mali, Cameroon, Nigeria, Kenya and South Africa (CABI, 1965; Daiber, 1971; James *et al*., 2010; Sæthre *et al*., 2011; Djomaha *et al*., 2016). Similarly, *B. brassicae* has been reported as key pests of cole crops in some African countries including Ethiopia, South Africa, Cameroon, Kenya, and Botswana (Amin and El-Defray, 1980; Bahana and Karuhize, 1986; Daiber, 1992; CABI, 1992; Munthali, 2009; Djomaha *et al*., 2016).

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For phytosanitary purposes and successful pest management, identification is of extreme importance. Aphid identification can be very difficult due to their morphological ambiguity and lack of physically obvious distinguishing characteristics (Miller and Footit, 2009; Li *et al.*, 2020). Additionally, intraspecific variation and phenotypic similarity can also impede aphid species identification (Li *et al*., 2020). *Lipaphis erysimi* occurs in both tropical and temperate regions and is occasionally conflated with the cabbage aphid, *B. brassicae* (Yue and Liu, 2000; Capinera, 2008). Their cornicles are both relatively short, but the body of *B. brassicae* is covered with a visible grayish waxy layer, which contrasts with a thin layer of white, waxy secretions on *L. erysimi* (Carter and Sorensen, 2013; Opfer and McGrath, 2013). As a result, the distribution and economic impact of the two brassica aphids are frequently misrepresented. It is thus very important to apply molecular identification tools such as DNA barcoding to morphological tools to further confirm aphid species. This study seeks to identify aphids present on cabbage, map their distribution, and understand their Spatiotemporal patterns in major cabbage-producing agroecological zones in Ghana.

3.2 MATERIALS AND METHODS

3.2.1 Study sites

Survey was done in the major cabbage growing regions of Ghana, namely Central, Eastern, Greater-Accra, Northern, Upper East, Volta, Ashanti, Bono, Bono East and Ahafo (MoFA, 2018), which cuts across five agroecological zones (Coastal Savannah, Deciduous Forest, Transition, Guinea savannah and Sudan savannah) (Table 3.1) from March, 2019 to December, 2020. Localities sampled were dependent on current information obtained from the Departments of Agriculture at the district level, on the presence of cabbage farms. A minimum of two districts was

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sampled per region with a minimum of three farms per district. The sampled locations with their geo-referenced positions (recorded using a GPS Coordinates app with a signal accuracy of 3-5m) are shown in Appendix 1. A total of 91 sites were sampled, as shown in Fig 3.1.

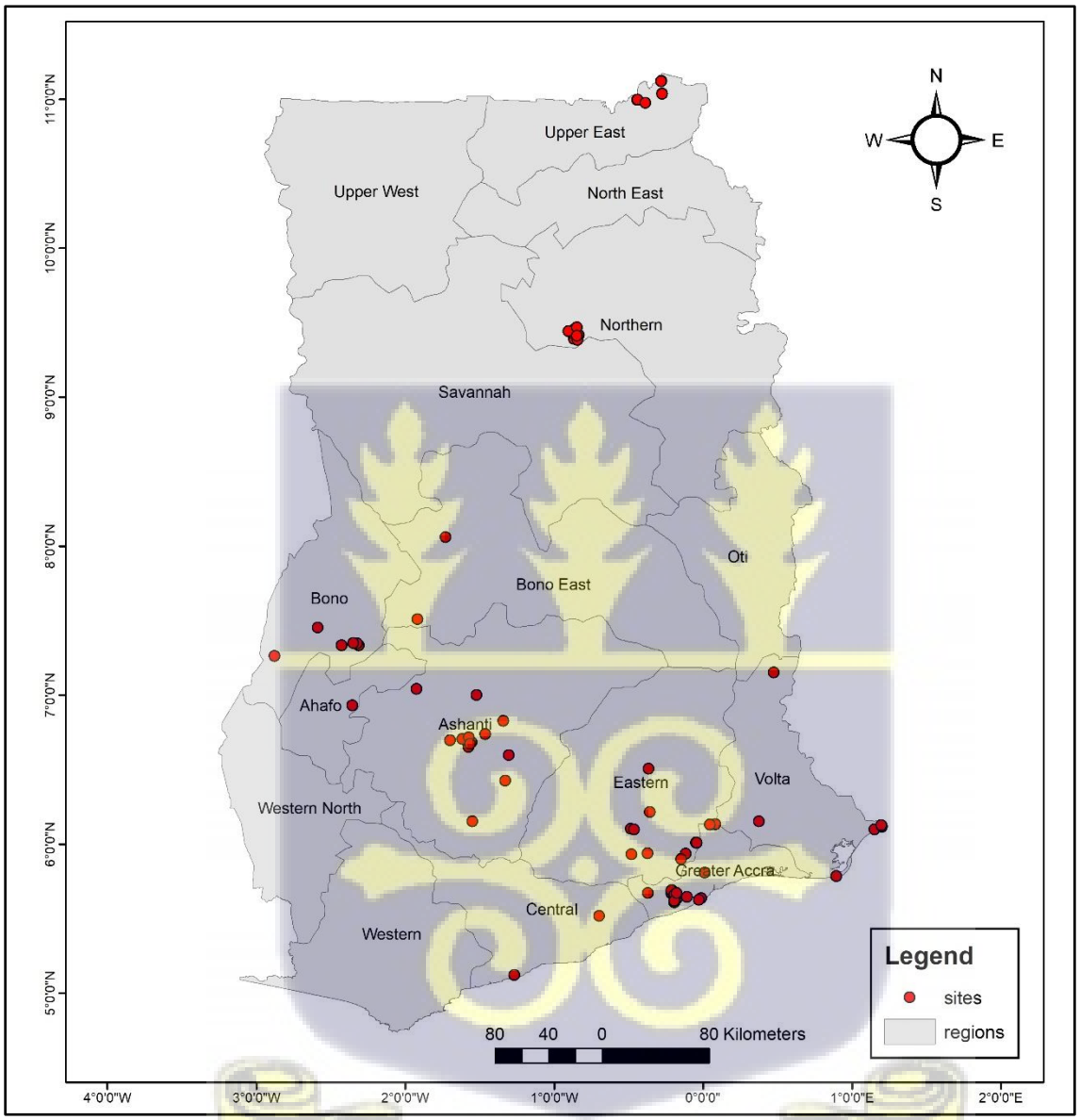


Figure 3-1: Study sites sampled for aphids during the study

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Table 3.1: Regions/Districts from which study sites were selected in Ghana

Region	Districts	Agroecological zone
Eastern	Akwapim North Suhum municipality Lower Manya New Juaben	Decidous Forest and Coastal Savannah
Greater Accra	Ga East Dzorwulu Tema Metropolitan	Coastal Savannah
Central	Agona West Municipal Cape Coast Metropolitan Awutu Senya East	Coastal Savannah and Deciduous Forest
Ashanti	Kumasi Metropolis Breman Asikuma Oforikrom	Deciduous Forest
Bono East	Techiman Municipal Kintampo	Deciduous Forest
Ahafo	Asutifi South	Transition
Bono	Sunyani West Sunyani Municipal	Transition
Volta	Ketu South South Tongu Keta	Coastal Savannah
Northern	Tamale Metropolitan Sagnarigu	Guinea Savannah
Upper East	Binduri Bawku Municipal Bawku West	Sudan Savannah

INTEGRI PROCEDAMUS

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3.2.2 Sampling for aphids

In each region, cabbage farms in the different targeted districts were selected for the survey depending on the availability. During the study period, trips were made to the selected sites when the cabbage crops were 2 weeks old or more. Sampling was done by randomly sampling 20 plants per farm following a 'Z' pattern. Three lower leaves of each plant were sampled and aphids on them were counted and recorded. Some aphids from the colonies of the sampled leaves were collected into vials containing 70% alcohol for species identification. Aphids were collected into different vials based on their colour (Green, Lemon-green and pink). Geo referenced points were noted for all farms where aphids were collected. The recorded number of aphids (mean number of aphids per plant) was used to calculate the abundance of identified aphids across the different sites sampled.

To determine the spatiotemporal variation of aphids across different agroecological zones, the density of aphids for the different regions was monitored monthly from April to October of 2019 and 2020 in one farm per district, for six of the seven regions sampled (excluding the Upper East region). During each sampling period, twenty (20) plants were randomly sampled in each farm by recording aphids on two lower leaves of each plant. To prevent the overestimation of aphids, destructive sampling modified from the method of Hughes (1963) and Forchibe *et al*. (2017) was used. Each leaf was harvested and placed in a bowl containing 70% alcohol to wash off aphids from the leaves. Camel hair brush was used to remove aphids still present on leaves after removing them from the alcohol. Aphids were counted and recorded, and monthly averages per plant was used to assess population density of aphids, across different agroecological zones. In agroecological zones within more than one region, the average was calculated and used as the average number of aphids count per plant for that zone.

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3.2.3 Identification of aphids

3.2.3.1 Morphological identification

The aphids collected from the field were mounted onto slides following the temporal insect mounting procedure as described by Blackman and Eastop (1984). The morphological examination was carried out with a Leica EZ4 D stereomicroscope at 40× magnification and the specimens were identified using the keys of Blackman and Eastop (1984). The major features for morphological separation are shown in Table 3.2.

Table 3.2: Major morphological distinguishing features of aphids on cabbage.

Feature	***M. persicae***	***L.e. pseudobrassicae***	***B. brassicae***
Frontal tubercles	Pronounced and converging	Diverging, not distinctly exceeding the vertex	Not developed
Cornicles	Cornicles are the same colour as the body and are long, > 2 times the length of the cauda	Cornicles are not dark and distinctly longer than the cauda	Relatively shorter and dark
Cauda	Cone-shaped	Tongue-shaped	Broadly triangular
Body colour	Pink, magenta, red, mid-green, pale-yellow green.	Yellowish green, grey-green, or olive green	Greyish green or dull mid-green
Body covering	Shiny	White waxy coating	Greyish-white mealy wax which gives it a grey colour

Source: Blackman and Eastop, 1984

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3.2.3.2 Molecular identification

Molecular identification of the aphids was done by sequencing the *Cytochrome c oxidase subunit I* (*CO-I*) gene at John Peter Carr's laboratory, Department of Plant Science, University of Cambridge. The procedure was done using the Thermo Scientific ™ Phire Plant Direct PCR Master Mix, designed to amplify DNA directly from samples.

a. DNA extraction and PCR

Five mg of a single aphid were placed into a microcentrifuge tube containing DNA extraction buffer and a micropestle was used to grind them to homogenise the mixture. The homogenate was spun for 20 seconds at a maximum speed of 7,000rpm. A 0.5µL of the supernatant was used for the PCR reaction. The PCR was carried out using the Phire Plant Direct PCR Master Mix, following the manufacturer's instructions. The universal primer pair HCO 2198 5'TAA ACT TCA GGG TGC CAA AAA ATCA-3' and LCO 1490 5'-GGT CAA CAA ATC ATA AAG ATA TTGG-3' were used to amplify an approximately 700 base pair DNA fragment of the mitochondrial gene (Folmer *et al*., 1994). The PCR cycling conditions were: initial denaturation at 95°C for 3 minutes followed by 30 cycles at 94°C for 30seconds, an annealing step at 50°C, an extension step at 72°C for 1 minute, and a final extension of 72°C for 10 minutes on a thermal cycler (ABI-Applied Biosystems, Veriti, USA).

b. Gel electrophoresis

The DNA amplicons generated by the PCR were loaded into wells of a 1% (w/v) agarose gel in TAE (Tris-Acid EDTA) buffer [0.04M Tris, 1mM EDTA pH 8.0, 0.1142% (v/v) glacial acetic acid] containing 0. 05µg.ml^{-1} ethidium bromide. The gels were submerged in TAE buffer and run in an MHU-1010 gel rig (Flowgen/Scientific Laboratory supplies, Yorkshire, UK) at 100V using

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a power-pac 3000 (Bio-Rad, Hemel Hempstead, UK). Ethidium bromide-stained gels were examined to reveal bands under UV illumination on an Alphalmager® gel documentation system (Alphannotech/ProteinSimple, Santa Clara, CA, USA) and depending on the expected amplicon size, an appropriate size marker (either 1kb ladder or 10kb ladder) was loaded adjacent to the samples.

c. Sequencing

The Sanger sequencing method was used after the electrophoresis. The gel positive PCR products were diluted to the required concentration specified by the sequencing provider (Source Biosciences Lifesciences, Nottingham, UK). All the amplicons were sequenced in both the forward and reverse directions using the respective forward and reverse primers used to generate them by using sequencing techniques developed by Sanger *et al.* (1977) and automated by Smith *et al.* (1986). Sequence identification was done by checking for homology using BLAST NCBI (Altschul *et al.,* 1990).

3.2.4 Pairwise comparison

A pairwise comparison was done to establish the closeness and identity of aphid species from Ghana to those from other places in the gene bank. The sequences generated through Sanger sequencing for the aphids and downloaded comparator sequences from GenBank (Appendix 2) were aligned using the MUSCLE algorithm and the percentage similarity computed using SDT software (Muhire *et al.,* 2014).

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3.2.5 Mapping of aphid distribution

Geo-referenced data points were used to plot the distribution of aphids identified during sampling. The regional abundance was calculated and plotted on pie charts to show the proportion of aphids identified per region.

3.2.6 Data analysis

Shapiro's test for normality and Levene's test for variance homogeneity were conducted on count data, to determine if they were normally distributed/homogenous. Data were not normally distributed nor homogenous and were further transformed using square root transformation. Transformed data were not normal nor homogenous, and were subjected to a non-parametric test; the Kruskal-Wallis *H* test (Kruskal and Wallis, 1952) to determine significance between the agroecological zones and months. Post-hoc mean separations were conducted using Bonferroni corrections of *p* values. All statistical analyses were performed in the R (version 3.5.1) environment for statistical computing (R Core Team, 2020).

3.3 RESULTS

3.3.1 Diversity of aphids on cabbage

Large colonies of green and pink aphids were observed on cabbage plants under the leaves examined at the study sites (Fig 3.2). The morphological examination distinguished them as *Lipaphis erysimi pseudobrassicae* and *Myzus persicae*. The key morphological characters used for distinguishing the mustard aphid (*L. e. pseudobrassicae*) from the green peach aphid (*M. persicae*)

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are as follows. Firstly, the aphid colonies possessed a thin layer of white waxy secretion (Fig. 3.3A). Microscopic observations of individual aphids showed that their cornicles (siphunculi) were not dark, and were longer than the cauda, the cauda was distinct and tongue-shaped, (Fig. 3.3A, 3.4A). For *M. persicae*, the cornicles had the same colour as the body; and were longer than 2 times the length of the cauda (Fig. 3.3B, 3.4B). Unlike *L. e. pseudobrassicae*, the cauda of *M. persicae* is cone-shaped (Fig. 3.3B, 3.4B). The antenna tubercles of *L. e. pseudobrassicae* are to be divergent while those of *M. persicae* are convergent (Figure 3.4A and 3.4B).

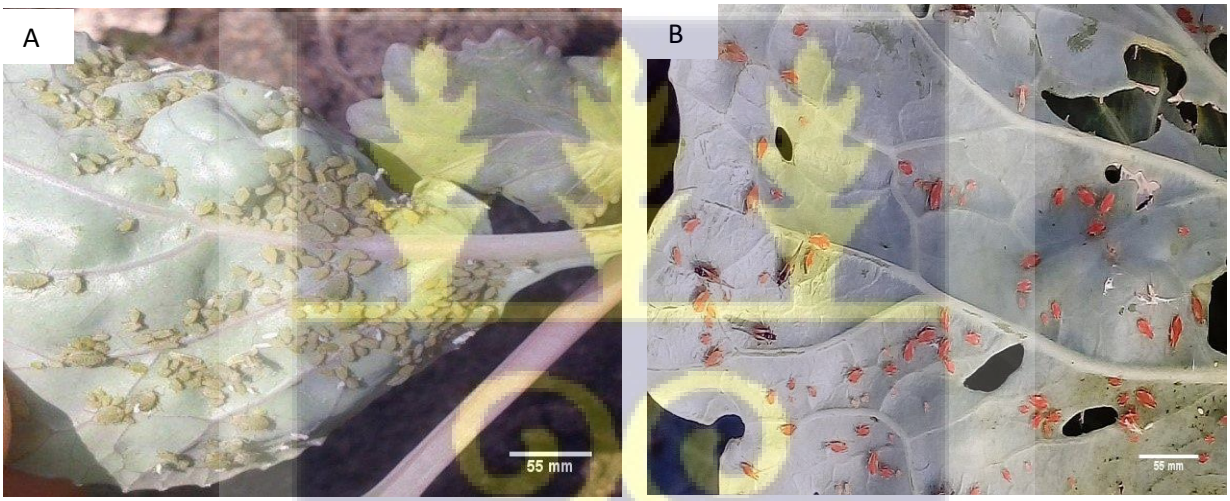


Figure 3-2: Colony of aphids on cabbage leaves A) L. *e. pseudobrassicae*, B) *M. persicae*.

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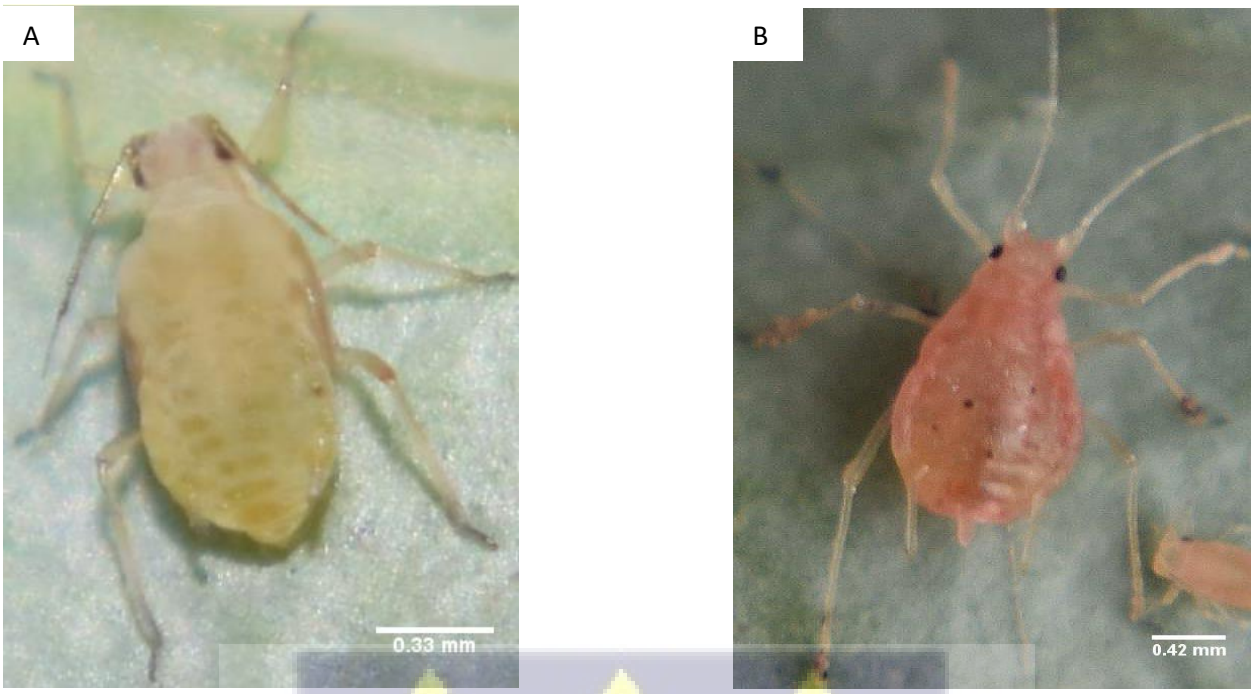
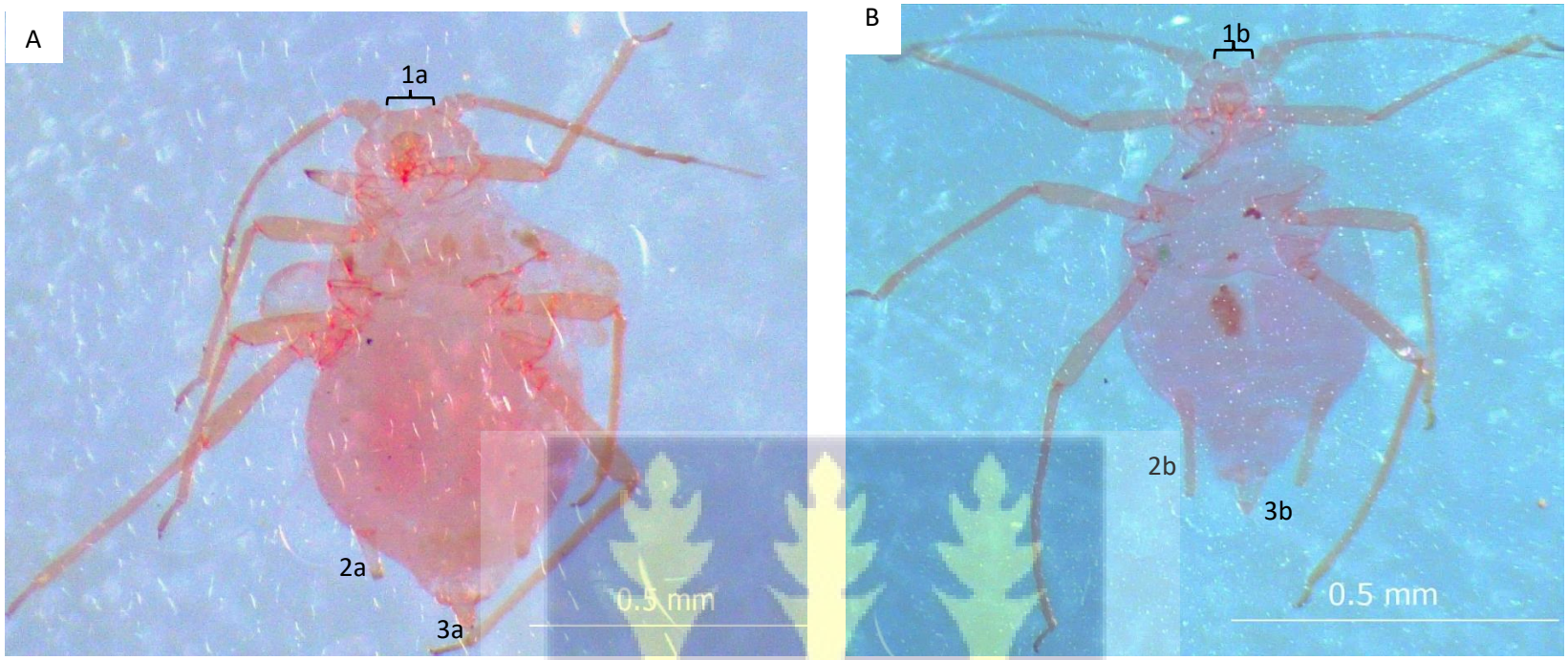


Figure 3-3: Aphids identified on cabbage in Ghana. A) *L. e. pseudobrassicae* (Davis 1814), B) *M. persicae* (Sulzer).

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Key

1a. Antenna tubercles of Lep diverging,

1b. Antenna tubercles of M. persicae converging,

3a. Cauda of Lep is tongue-shaped

3b. Cone-shaped cauda of M. persicae

2a. Cornicles of Lep as long as cauda,

2b. Cornicles of M. persicae 2x longer than cauda

Figure 3-4: Distinguishing features of *L. e. pseudobrassicae* (A) and *M. persicae* (B) as seen under the Leica EZ digital microscope (X35 Mag.).

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The molecular analysis confirmed the morphological identification as *Lipaphis erysimi pseudobrassicae* and *Myzus persicae*. The DNA barcoding gene, Cytochrome Oxidase subunit I (COI) aligned at the 700bp fragment (Fig. 3.5), which is typical of aphids.

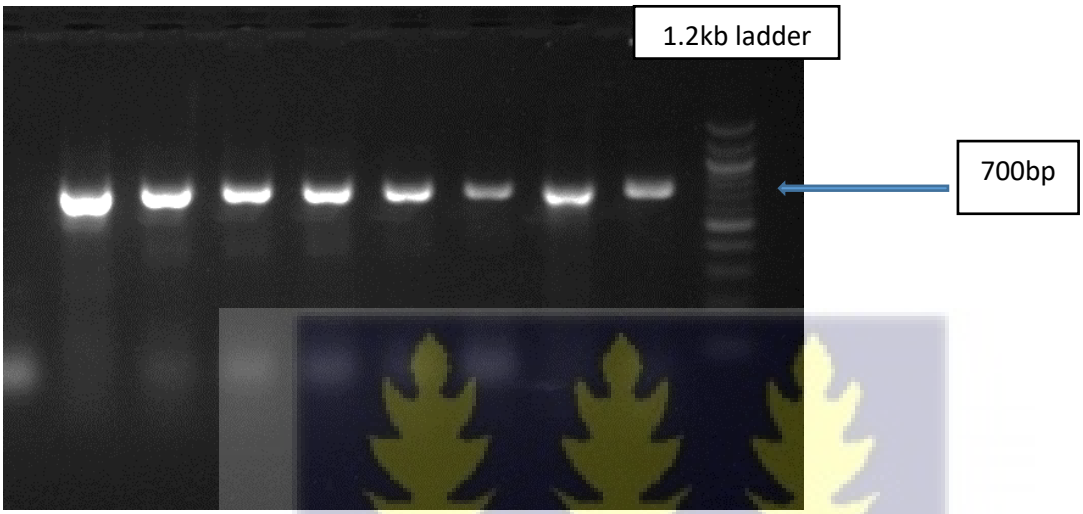


Figure 3-5: Gel electrophoresis showing 700bp fragment of 5' region of the mitochondria *cytochrome oxidase I – (CO-I)* gene for identification of aphid species.

Of the 95 samples analyzed 35 were *M. persicae* and 60 were *L. e. pseudobrassicae*. The BLAST search for homology using the generated sequences; revealed a 95-100% sequence similarity to *L. e. pseudobrassicae* and *M. persicae* isolated from cabbage plants in Bangladesh, Benin, Belarus, Japan and India (Appendix 2).

The pairwise sequence comparison showed a clear species demarcation between *L. e. pseudobrassicae* and *M. persicae* from reference sequences from GenBank (Fig. 3.6)

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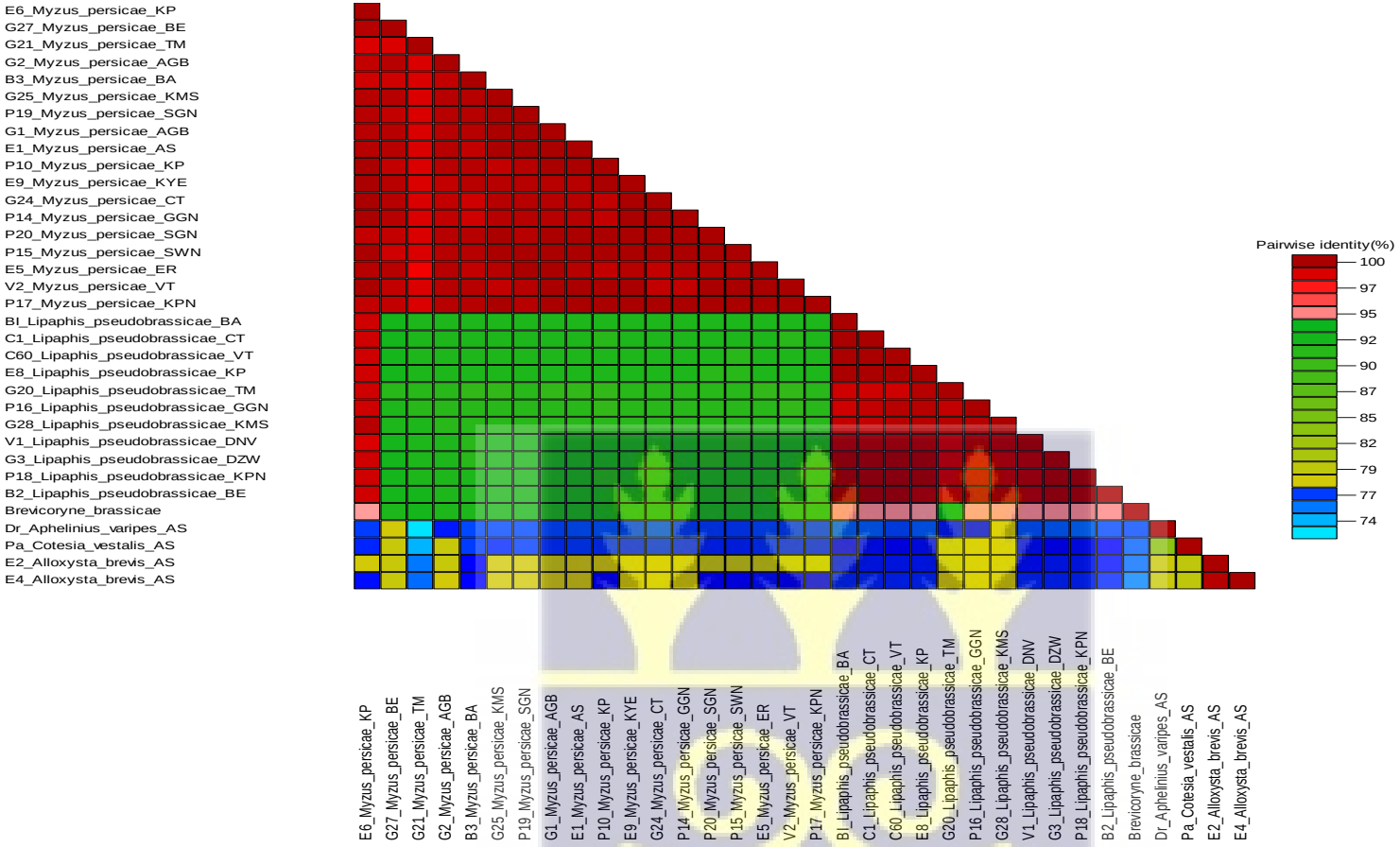


Figure 3-6: Pairwise comparison of CO1 gene sequences for *Lipaphis erysimi pseudobrassicae* and *Myzus persicae.*

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3.3.2 Distribution and abundance of aphids on cabbage in Ghana

A total of 91 sites were visited spanning across the Northern and Southern parts of Ghana with the most observations found in the South (Fig 3.7). *Lipaphis e. pseudobrassicae* and *M. persicae* were recorded in 89 and 76 locations respectively. Both species of aphids, *L. e. pseudobrassicae* and *M. persicae* were found co-occurring on cabbage at 74 out of the 91 locations, but with the former having large numbers (Fig 3.7). Aphids were found to have a heterogenous distribution, with few sites having high aphid populations and most with low populations. Part of the distribution has been published in Fening *et al*. (2020)[1].

Generally, the mean number of aphids per plant collected was small throughout the survey periods. Nevertheless, *L. e. pseudobrassicae* was found to be the more dominant and more abundant aphid species in all the sites compared to *M. persicae* (Fig. 3.7).

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[1] Fening, K. O., Forchibe, E. E., Wamonje, F. O., Adama, I., Afreh-Nuamah, K., & Carr, J. P. (2020). First report and distribution of the indian mustard aphid, *Lipaphis erysimi pseudobrassicae* (Hemiptera: Aphididae) on cabbage (*Brassica oleracea* var capitata) in Ghana. *Journal of Economic Entomology*, *113*(3), 1363-1372.

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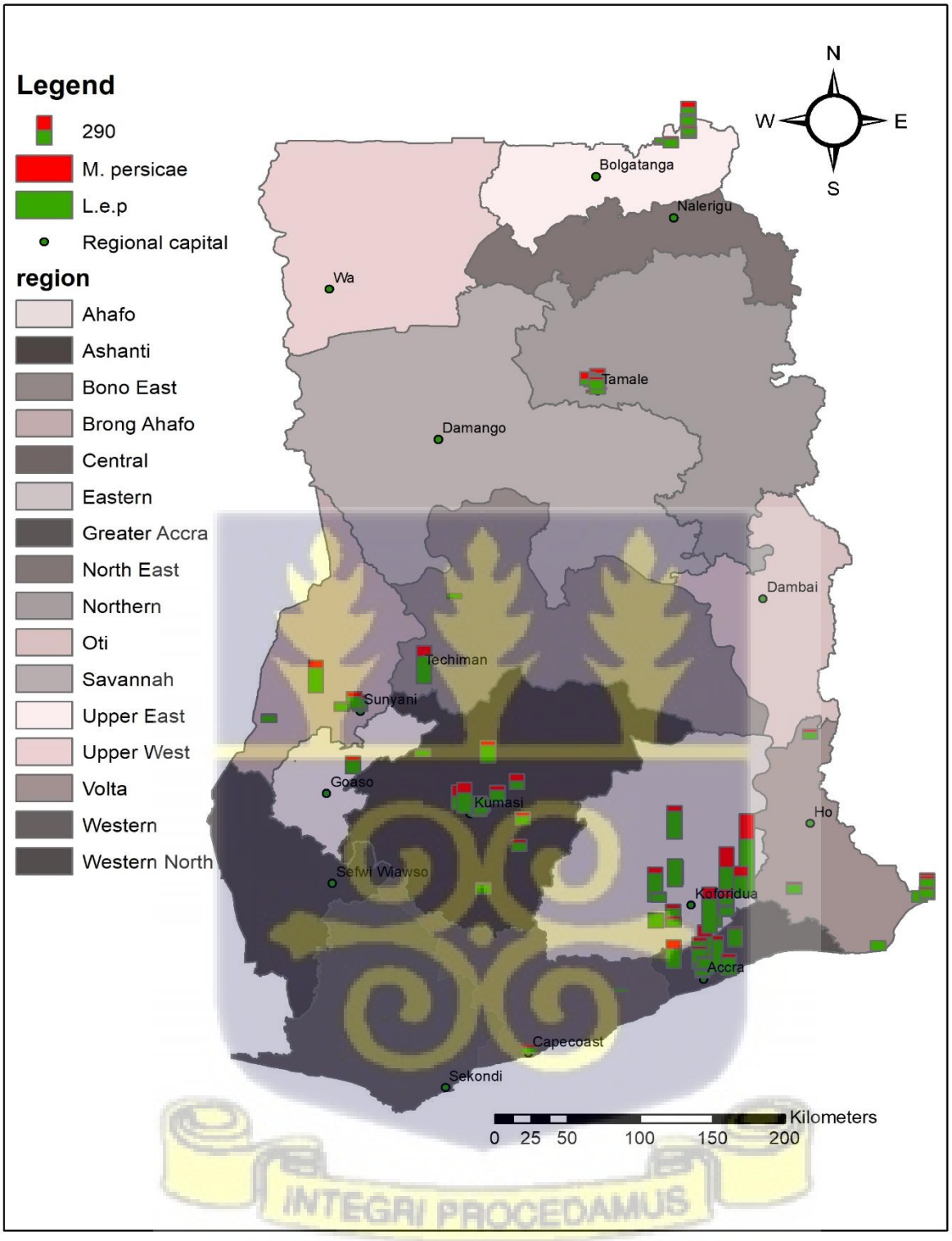


Figure 3-7: Distribution and abundance of *Lipaphis erysimi pseudobrassicae* and *Myzus persicae.*

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3.3.3 Spatiotemporal variation of *L. e. pseudobrassicae* population across four agroecological zones

The spatiotemporal variation of *L. e. pseudobrassicae* from April – October in 2019 and 2020 across four agroecological zones are shown in Figure 3.8. The Deciduous Forest agroecological zone had the highest peaks of *L. e. pseudobrassicae* in both 2019 and 2020 (Fig 3.8) followed by the Coastal Savannah zone in 2019 (Fig. 3.8A). The smallest populations were recorded in the transition zone in 2019 (Fig. 3.8A). In the case of the Deciduous Forest zone, there was a steady build-up of the population in 2019 and the average peak was recorded in September, and then a sharp decline in October (Fig. 3.8A). Kruskal-Wallis H test showed a significant difference in *L. e. pseudobrassicae* density between the agroecological zones for 2019 in April (χ^2 (3) = 9.359, p = 0.024), May (χ^2 (3) = 9.667, p = 0.021), June (χ^2 (3) = 10.120, p = 0.017), July (χ^2 (3) = 9.596, p = 0.022), August (χ^2 (3) = 9.359, p = 0.024), (Table 3.3). No significant difference was recorded in September (χ^2 (3) = 5.769, p = 0.123) and October (χ^2 (3) = 2.097, p = 0.552) (Table 3.3).

In 2020, the spatio-temporal variation in each of the agroecological zones had different density trends. However, the peak population density was recorded in July for all agroecological zones (Fig. 3.8B). The largest average population density was recorded in the Deciduous Forest agroecological zone, while the least was recorded in the guinea savannah agroecological zone (Fig. 3.8B). There were no significant differences in *L. e. pseudobrassicae* density between agroecological zones for 2020 in April (χ^2 (3) = 6.995, p = 0.072) and August (χ^2 (3) = 6.282, p = 0.098). (Table 3.3). Significant mustard aphid density differences were recorded for May (χ^2 (3) = 9.596, p = 0.022), June (χ^2 (3) = 8.243, p = 0.041), July (χ^2 (3) = 9.154, p = 0.027), September (χ^2 (3) = 8.128, p = 0.043), and October (χ^2 (3) = 9.974, p = 0.018). Differences between means are shown in table 3.3.

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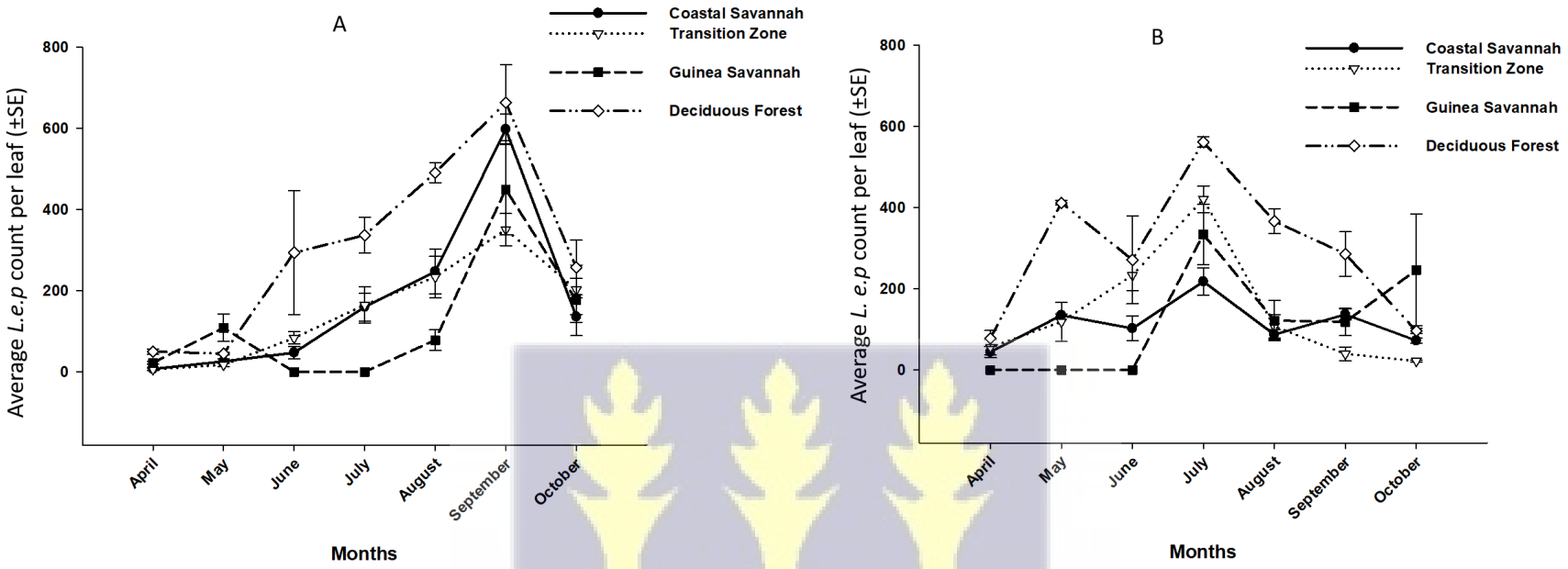


Figure 3-8: Mean number of *Lipaphis erysimi pseudobrassicae* across four agroecological zones of Ghana, from April to October 2019 (A) and 2020 (B).

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3.3.4 Spatiotemporal variation of *M. persicae* across four agroecological zones

The spatiotemporal variation of *M. persicae* in each of the agroecological zones had different fluctuation trends. Fig. 3.9 shows the spatiotemporal density trend for 2019 and 2020. In 2019, the population peaked in September and July for the Deciduous Forest and Coastal Savannah agroecological zones respectively. In the transition zone, the peak populations were recorded in October and June, while in the Guinea savannah zone, the peak populations were recorded in May and September (Fig. 3.9A). The largest population densities were recorded from June to August for the Transition and Guinea Savannah zones, and May to April for the Deciduous Forest and Coastal Savannah zones (Fig 3.9A). Kruskal-Wallis H test showed a significant difference in *M. persicae* density between agroecological zones in 2019, for April (χ^2 (3) = 9.312, p = 0.025), May (χ^2 (3) = 8.078, p = 0.044), June (χ^2 (3) = 8.868, p = 0.031), July (χ^2 (3) = 10.16, p = 0.017), August (χ^2(3) = 7.931, p = 0.047), and September (χ^2 (3) = 8.321, p = 0.041) (Table 3.3). No significant difference in *M. persicae* density between agroecological zones was recorded in October (χ^2 (3) = 6.897, p = 0.075) (Table 3.3).

M. persicae population density was low in 2020, nevertheless, the highest population was recorded in the Deciduous Forest agroecological zone (Fig. 3.9B). The population density was low from April to June for all agroecological zones. The peak population density was recorded in October for the Deciduous Forest and guinea savannah zones (Fig. 3.9B). There were no significant differences in *M. persicae* aphid density between agroecological zones July (χ^2 (3) = 6.527, p = 0.088), August (χ^2 (3) = 6.55, p = 0.087) and September (χ^2 (3) = 6.291, p = 0.098). Nevertheless, there were significant differences among agroecological zones in April (χ^2 (3) = 9.312, p = 0.025), May (χ^2 (3) = 9.18, p = 0.026), June (χ^2 (3) = 8.39, p = 0.038), and October (χ^2 (3) = 8.641, p = 0.034) (Table 3.3). Differences between *M. persicae* population means are shown in table 3.3.

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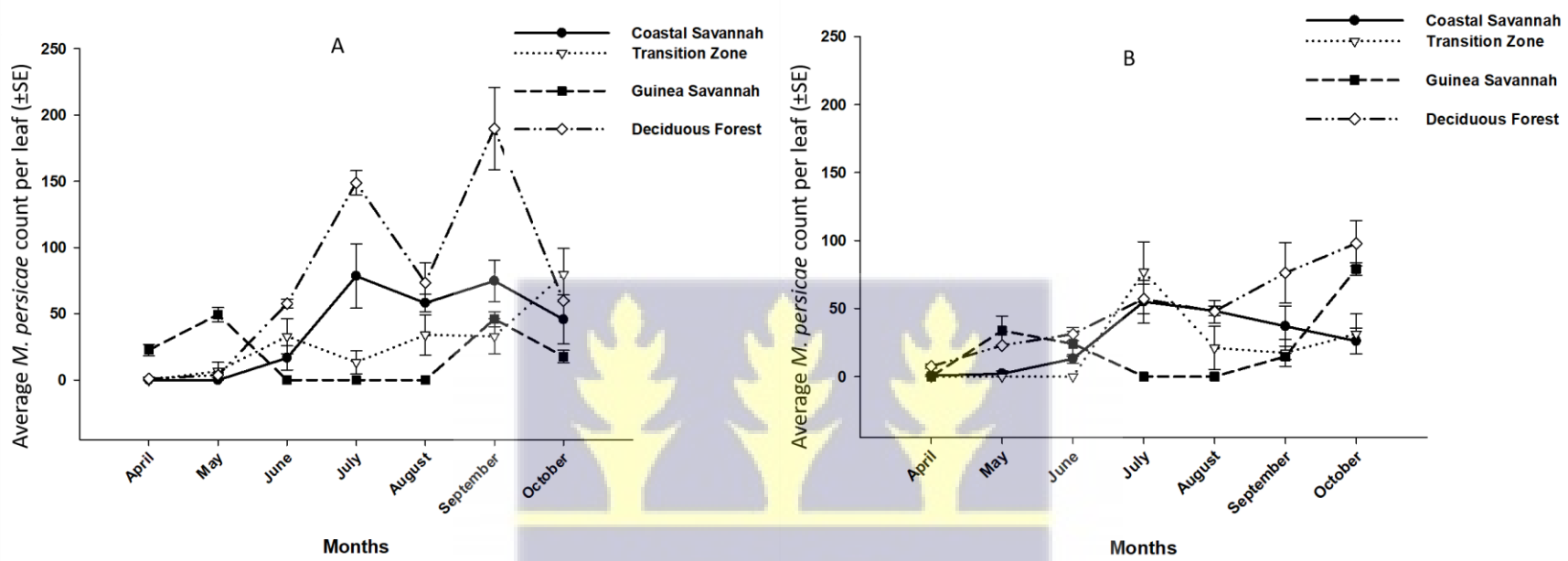


Figure 3-9: Mean number of *Myzus persicae* across four agroecological zones of Ghana, from April to October 2019 (A) and 2020 (B).

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Table 3.3: Occurrence of aphids and their mean (±SE) population per leaf in each agroecological zone.

Year	Period	Species	Agroecological zone			
			Coastal Savannah	Deciduous Forest	Guinea Savannah	Transition
2019	April	*L. e. p*	7.00 ± 3.28bc	50 ± 5.94a	22 ± 3.71ab	6.00 ± 3.21c
		M. persicae	0 ± 0b	1.00 ± 0.58b	23 ± 13.09a	0 ± 0b
	May	*L. e. p*	26 ± 5.73bc	45 ± 2.80ab	108 ± 33.65a	17 ± 3.79c
		M. persicae	0 ± 0b	4.00 ± 2.12ab	49 ± 28.48a	7.00 ± 3.84ab
	June	*L. e. p*	47 ± 15.88bc	294 ± 53.52a	0 ± 0c	83 ± 14.99ab
		M. persicae	17 ± 9.62ab	58 ± 33.29a	0 ± 0b	33 ± 19.05a
	Jul	*L. e. p*	160 ± 34.61bc	337 ± 43.84a	0 ± 0c	165 ± 45.17ab
		M. persicae	78 ± 45.29a	149 ± 85.93a	0 ± 0b	13 ± 7.70b
	August	*L. e. p*	247 ± 55.54ab	491 ± 24.44a	78 ± 25.32c	233 ± 50.93bc
		M. persicae	58 ± 33.55a	74 ± 42.44a	0 ± 0b	34 ± 19.63ab
	September	*L. e. p*	598 ± 37.57a	663 ± 93.4a	449 ± 112.32a	350 ± 39.59a
		M. persicae	75 ± 43.17ab	190 ± 109.50a	46 ± 26.56b	33 ± 19.05b
	October	*L. e. p*	136 ± 46.53a	257 ± 67.30a	176 ± 54.37a	202 ± 61.08a
		M. persicae	46 ± 26.37ab	60 ± 34.54ab	18 ± 10.20b	80 ± 45.99a
2020	April	*L. e. p*	44 ± 13.23a	78 ± 19.75a	0 ± 0a	56 ± 18.52a
		M. persicae	1.00 ± 0.67b	8.00 ± 1.61a	0 ± 0b	0 ± 0b
	May	*L. e. p*	135±3.39ab	412 ± 5.48a	0 ± 0c	118 ± 47.80bc
		M. persicae	2.00 ± 2.00b	23 ± 0.44a	34 ± 10.71a	0 ± 0b
	June	*L. e. p*	102 ± 30.21ab	271 ± 18.27a	0 ± 0b	233 ± 37.68a
		M. persicae	13 ± 3.09ab	31 ± 4.89a	24 ± 9.17a	0 ± 0b
	Jul	*L. e. p*	217 ± 33.75c	561 ± 12.84a	334 ± 74.62bc	421 ± 32.84ab
		M. persicae	55 ± 15.89a	57 ± 10.82a	0 ± 0a	77 ± 22.11a
	August	*L. e. p*	87 ± 11.85a	366 ± 30.22a	121 ± 49.46a	107 ± 29.02a
		M. persicae	48 ± 3.47a	48 ± 8.29a	0 ± 0a	21 ± 15.82a
	September	*L. e. p*	137 ± 13.72ab	286 ± 54.56a	118 ± 33.72ab	39 ± 7.25b
		M. persicae	37 ± 14.61a	76 ± 22.26a	15 ± 2.40a	17 ± 9.82a
	October	*L. e. p*	72 ± 5.88bc	96 ± 5.89ab	246 ± 37.50a	22 ± 2.73c
		M. persicae	26 ± 9.45b	98 ± 16.66a	79 ± 4.58ab	31 ± 14.62b

L.e.p = Lipaphis erysimi pseudobrassicae, means with different letters are significantly different across rows.

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3.4 DISCUSSION

3.4.1 Diversity and distribution of aphids on cabbage

Two major aphids were found in cabbage in all regions; *Lipaphis erysimi pseudobrassicae* and *Myzus persicae*. *L. e pseudobrassicae* was first reported in Ghana in 2016 (Fening *et al*., 2016), and is a specialist aphid on brassicaceous crops, while *M. persicae* is a polyphagous and cosmopolitan species and is found on several crops around the world (CIE, 1979; Millar, 1994). Two morphologically similar but clearly distinct geographically separated forms or subspecies of the mustard aphids are known to occur; namely the European form, *Lipaphis erysimi erysimi* and *Lipaphis erysimi pseudobrassicae* which constitutes the Indian strain (Müller, 1986; Ronquist and Ahman, 1990). Until this classification, *L. e. pseudobrassicae* was referred to as *L. pseudobrassicae* (Davis) and *L. e. erysimi* was referred to as *L. erysimi* (Kaltenbach) (Müller, 1986). Of the two forms, *L. erysimi pseudobrassicae*, is considered a noxious insect to crops (Müller 1986). Studies have attributed widespread infestation of cabbage in warmer countries to *L.erysimi pseudobrassicae* (Davis) (Daiber, 1971; James *et al*., 2010; Sæthre *et al*., 2011; Djomaha *et al*., 2016).

Farms sampled during the survey ranged from backyard gardens to large commercial farms. The majority of the farmers cultivated cabbage in urban and peri-urban areas. Obuobie *et al.* (2006) reported similar findings; most exotic leafy vegetables are grown in and around urban areas to meet the ever-increasing urban demand. In all the regions surveyed, *L. e pseudobrassicae* was present and most abundant in sites where it co-existed with *M. persicae* suggesting that all the regions have suitable habitat for the establishment of *L. e pseudobrassicae*. This further suggests that this pest is highly adaptable to different types of environments. This finding is congruent with

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studies by Souza *et al.* (2017) which indicated that *L. pseudobrassicae* is able to adapt to changing temperatures. The highest abundance of *L. e. pseudobrassicae* in southern Ghana could be because this area has long been growing exotic leafy vegetables such as cabbage, and has a much more suitable climate for its development and spread.

The Southern sector of Ghana is characterized by a bimodal rainfall regime while the Northern sector experiences a unimodal rainfall regime (Agbessenou *et al*., 2016), which accounts for the year-round cultivation of cabbage in the Southern sector. The cabbage growing periods also vary significantly between the Northern and Southern sectors of the country. In the North, most farmers grow cabbage during the dry season as earlier reported by Timbilla and Nyarko (2004). In addition, informal interactions with farmers in the North showed that most farmlands for cabbage cultivation are flooded during their single rainy season, and so prevent year-round cultivation. Since the establishment and growth of aphid populations are dependent on environmental factors such as temperature, relative humidity and rainfall, their absence in certain areas could be attributed to this effect and partly to the management regimes on farmer's fields.

3.4.2 Spatiotemporal variation of aphids

Species abundance and spatiotemporal distributions are often affected by agroecological barriers, usually characterized by differences in physical environments (Shukla, 1990; Faheem *et al*., 2019). In this study, the highest abundance of *L. e pseudobrassicae* and *M. persicae* was recorded in the Deciduous Forest and Coastal Savannah agroecological zones. This could be attributed to the fact that more cabbage is grown in Southern Ghana, as seen in the high distribution sites recorded.

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Additionally, these agroecological zones are home to the biggest cities in Ghana, which have a high demand for exotic vegetables such as cabbage (Obuobie *et al*., 2006; Wongnaa *et al*., 2019; Quansah *et al*., 2020). The Deciduous Forest agroecological zone is characterized by a warm and moist climate, frequent rainfalls, high vegetative diversity, and very fertile soils that favour the growth of food crops, hence suitable for insect diversity (Kemausour *et al*., 2013). The Coastal Savannah agroecological zone is characterized by warm temperatures all year round with fertile soils and fewer rainfall days; however, urbanization has led to increasing urban and peri-urban vegetable farming to supply the ever-growing demand (Timbilla and Nyarko, 2004; Kemausour *et al*., 2013). This greatly accounts for the high abundance of aphids recorded in these zones. Low abundance of aphids recorded in the Guinea savannah and transition zones could be attributed to the fact that these zones are characterized by less amount of rainfall due to unimodal rainfall patterns, and prolonged droughts which make it difficult to grow cabbage without reliable supplementary irrigation (Ackah and Kotei, 2021). For example, Maggio *et al*. (2005) reported that drought stress decreased the total yield of cabbage by approximately 70%. Fewer farmers grow cabbage in the urban areas, however, most farmers practice bush fallowing, growing more drought-tolerant crops such as sorghum and millet (Wood, 2013). These findings are consistent with Faheem *et al*. (2019), who found variations in the abundance of wheat aphids across different agroecological zones.

The spatial variation of aphids can be predicted by climatic factors and other biotic factors such as vegetation, host plants, natural enemies and migration (Faheem *et al*., 2019). The spatial variation of both aphids showed that high densities were recorded from July to September of 2019 and in July in 2020 across all agroecological zones while the lowest was recorded from April to June in

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2019 and in June in 2020. Asare-Nuamah and Botchway (2019) reported that April to June is characterized by high rainfall patterns, with June being the wettest month in the Deciduous Forest, Coastal Savannah and transition zones. This could have accounted for the low aphid populations recorded in June across the different agroecological zones, although not covered in the scope of the study. Additionally, low aphid populations were also recorded in August and October in the Guinea savannah zone in both years, which concurs with data reported by Asare-Nuamah and Botchway (2019) that, the highest rainfall occurs from August to October in this zone, also known to be the coolest months.

Conclusively, two aphid species; *L. e. pseudobrassicae* and *M. persicae* occur on cabbage in Ghana, with *L. e. pseudobrassicae* being the most abundant. Their distribution was clustered in the South of the country due to the presence of higher number of cabbage farms in these parts compared to the Northern parts. The spatiotemporal variation differed significantly between the four agroecological zones studied, with higher aphid densities recorded in the Deciduous Forest and Coastal Savannah zones.

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CHAPTER 4

4.0 Spatial patterns and habitat suitability modelling of *L.e. pseudobrassicae* and *M. persicae* on cabbage in Ghana.

4.1 INTRODUCTION

Drastic changes in temperature, precipitation, humidity and other environmental factors caused by global climate change, have heightened the likely incidence of pests and diseases, threatening agricultural production (Strange and Scott, 2005). Remote detection of correlations between pest occurrence and potentially suitable habitats is important for limiting economic losses due to crop damage, pesticide overuse and subsequent environmental pollution (Gooshbor *et al*., 2016; Yuan *et al*., 2017). Spatial Statistics tools have been applied in agriculture to help detect and model spatial links and correlations between the presence, absence and density of pests in response to climatic, environmental, and human factors (AL-Kindi *et al*., 2017).

Mapping insect spatial pattern is important to understand their movement into and within crops (Ferguson *et al*., 2000), to underpin the development of pest management strategies in such a way that pesticide applications may be spatially targeted to minimize their negative impacts (Evans *et al*., 1998; Ferguson *et al*., 2003). A spatial pattern can be classified as random, uniform, or aggregate. Random patterns occur when the probability of insect infestation on all plants is constant and independent, while aggregate patterns are usually associated with little or no insect mobility. Uniform patterns rarely occur in nature unless it is induced, where individuals are equally spaced apart. Indices such as David and More index, the Taylor power law, and the Lloyd and Iwao patchiness, among others have been used in insect distribution studies, where the relation between the mean and variance was used to determine the distribution pattern (Ruiz-Cárdenas *et*

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al., 2003; Ribeiro *et al*., 2009). However, these indices do not take into consideration the spatial occurrence of the studied samples, thus, limited in describing spatial patterns (Ribeiro *et al*., 2009). Spatial statistical tools have been used to map insect and disease spatial patterns, as seen in Lessio *et al*. (2011) who used geostatistical tools and artificial neural networks to map the spatial pattern of *Scaphoideus titanus*, a disease vector of grapevine in Italy, Aziz *et al*. (2012) used the Clark and Evans Nearest Neighbor statistics to determine the spatial pattern of dengue in Kuala Lumpur.

Ecological modeling has been used to understand and predict insect behaviour, thus supports decision-making for pest management (DŲeroski, 2009). Typically, the major aspects modelled are population dynamics of interacting species or the habitat-suitability for particular species, which are often constructed from measured data using machine learning (DŲeroski, 2009). Habitat-suitability modelling studies the effect of spatially varying abiotic factors of a habitat on the presence, abundance or diversity of a given organism. Machine learning-based models that have been successfully applied in ecological modelling include Maxent (Maximum entropy) (Phillips *et al*., 2006), artificial neural networks (Watts and Worner, 2006), Random Forest (RF) (Cutler *et al*., 2007), among others. In Sultana *et al*. (2017) Maxent was used to map the habitat suitability of *Bactrocera tryoni* worldwide, Fiaboe *et al*. (2012) predicted the worldwide distribution of *Rhynchophorus ferrugineus* using Maxent, Cuéllar *et al*. (2020) modelled the abundance of culicoides biting midges using Random Forest machine learning. Pest distribution models can make significant contributions to management guidelines (Ascough *et al*., 2008) by providing baseline information on pest adaptations, location-based trends/patterns and spread, to guide target-specific management implementations. Given the current changing climatic patterns, species habitat suitability models can be used to predict current and future suitable habitats under changing climatic conditions (Biber-Freudenberger *et al*., 2016).

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Lipaphis erysimi pseudobrassicae and *M. persicae* are a huge problem to cabbage production in Ghana (Forchibe *et al*., 2017). Large infestations are often associated with a necrotic disease which leads to 100% damage (Adenka *et al*., 2021), and as such farmers resort to extensive use of pesticides for their control (Baidoo *et al*., 2012). Understanding aphid spatial patterns and potential habitat suitability will provide baseline data for predicting future aphid occurrence and distributions, relationships between key climatic factors favouring aphid occurrence, and possible farming areas with low aphid incidence. This study sought to elucidate the spatial distribution of *L. e. pseudobrassicae* and *M. persicae* on cabbage, using survey data from Chapter 3, which comprised of georeferenced points with different aphid infestation levels on cabbage at each point. The study aims to understand the spatial pattern of these two aphids and assess a species distribution model (SDM) for their potential habitat suitability in Ghana.

4.2 MATERIALS AND METHOD

4.2.1 Aphid occurrence data

Occurrence data for *L. e. pseudobrassicae* and *M. persicae* were collected during a field survey as reported in section 3.2.1 of chapter 3. The map for the sites visited is shown in Fig. 3.1

4.2.2 Point Pattern Analysis

To determine the spatial pattern of aphids' distribution, if aphids were clustered, random, or uniformly distributed, the point pattern Average Nearest Neighbour (ANN) algorithm in Argis Pro was used. The Average Nearest Neighbour analysis was suggested by Clark and Evans (1954), as a method to analyze the spatial pattern of animals/plants by measuring the distance from an

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individual to its nearest neighbour. It calculates the distance between each aphid occurrence point and its nearest neighbour and then finds the average of all the nearest neighbour distances known as the Average Nearest Neighbour Distance (D_0).

$$D_O = \frac{\sum_{i=1}^{n} d_i}{n}$$

It also calculates the Average Nearest Neighbour distance of a hypothetically random distribution (D_E) based on the number of observations and Area.

$$D_E = \frac{0.5}{\sqrt{(n/A)}}$$

Where n is the number of observations and A is the minimum area that would enclose all occurrence data points. The ratio of D_0 to D_E (R) is then used to determine the spatial pattern of the points.

$$R = \frac{D_O}{D_E}$$

Where, D_0 is the observed mean distance between each aphid occurrence point and its nearest neighbor and D_E is the hypothetical average nearest neighbor distance.

Generally, if R is less than 1 ($R < 1$), it implies that the observed nearest neighbour distances were much closer to each other than that of the hypothetical random distribution resulting in a clustered spatial pattern. If R is greater than 1 ($R > 1$), it implies is that the observed nearest neighbour distances were much larger than that of the hypothetical random distribution resulting in a dispersed spatial pattern. If R equals 1 ($R = 1$), the implication is that the observed nearest neighbour distances are the same as that of the hypothetical random distribution hence the spatial

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pattern would be considered as random. The Average Nearest Neighbour tool in ArcGIS Desktop was used to determine the spatial pattern of the insects by implementing Euclidean Distance as the straight-line distance between any two given points. The standard deviation z-score shown in the equation below was used to confirm the significance of the observed pattern was calculated as (Clark and Evans, 1954):

$$z = \frac{\bar{D}_O - \bar{D}_E}{SE}$$

Where, $SE = \frac{0.26136}{\sqrt{n^2/A}}$ is the standard error of the mean, in which the constant is derived from the radius of a circle of unit area and the Poisson probability model. Average nearest neighborhood analyses were done separately for each aphid species. The results of the spatial pattern analysis were visualized using the Point Density analysis tool in ArcGIS Desktop, which calculates the number of points in an area and creates a surface for the visualization.

4.2.3 Environmental variables

To model aphid habitat suitability, 19 bioclimatic variables were applied (derived from temperature and precipitation measures), averaged between 1970 and 2000 at global coverage, with a spatial resolution of 30 arcsec (~1 × 1 km), from the WorldClim 2.1 database (http://www.worldclim.org/, Accessed: 1 August 2020). The bioclimatic variables were computed from monthly values of minimum, average, and maximum temperature and monthly precipitation, and each variable was clipped to the shape of Ghana using the 'extract by mask tool' in ArcGIS Desktop (ESRI Inc., http://www.esri.com/). The variables used in the analysis included the following (Table 4.1) and the visualizations are shown in Appendix 4:

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Table 4.1: Bioclimatic variables used in the model

ID	Bioclimatic Variable
BIO1	Annual Mean Temperature
BIO2	Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO3	Isothermality (BIO2/BIO7) (×100)
BIO4	Temperature Seasonality (standard deviation ×100)
BIO5	Max Temperature of Warmest Month
BIO6	Min Temperature of Coldest Month
BIO7	Temperature Annual Range (BIO5-BIO6)
BIO8	Mean Temperature of Wettest Quarter
BIO9	Mean Temperature of Driest Quarter
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO13	Precipitation of Wettest Month
BIO14	Precipitation of Driest Month
BIO15	Precipitation Seasonality (Coefficient of Variation)
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter
BIO18	Precipitation of Warmest Quarter
BIO19	Precipitation of Coldest Quarter

Source: WorldClim 2.1 database (http://www.worldclim.org/, Accessed: 1 August 2020)

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4.2.4 Species distribution modelling

A predictive analysis was performed to infer the underlying processes that contributed to the observed spatial distribution patterns in the density maps. To determine the driving factors, *L. e. pseudobrassicae* and *M. persicae* were modelled separately using the Random Forest package in the R statistical and programming environment. Random Forest (RF) modelling tool is a powerful tree-based supervised machine learning algorithm that automatically learns the relationships between a set of predictors and a target variable and then predicts the outcome with a new set of predictors (Cutler *et al*., 2007; Zhang *et al*., 2014). In RF species modelling, absence data is as important as presence data hence in species distribution modelling where only presence data are collected, a set of locations where it is unlikely to find a species are used to represent absence data also known as pseudo-absence data (Elith *et al*., 2006; Barbet-Massin *et al*., 2012; Zhang *et al*., 2014). To account for locations where no species were found, an equal number of presence points were generated separately at random to serve as absence data for the *L. e. pseudobrassicae* and *M. persicae* such that the locations do not fall on already surveyed points. This was necessary to maintain a presence to absence ratio of 1:1 in order to achieve an unbiased model and the best modelling accuracy. The species distribution modelling package (dismo) (Hijmans *et al*., 2017) in the R environment was used in the generation of the random points. This allows the response variable to have at least two outcomes to be modelled for machine learning.

The presence and absence data were combined into one dataset to represent the response variable where occurrence was indicated as ones and pseudo absence was indicated as zeros. The dataset was then overlayed on a stack of all bioclimatic variables to create a regression matrix ready for machine learning training.

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4.2.5 Evaluation of model performance.

This was performed separately for *L. erysimi pseudobrassicae* and *M. persicae* datasets. The Classification and Regression Training (caret) R package were used to initially determine the best configuration for the Random Forest model using a 10-fold block cross-validation process of model training and testing (Roberts *et al*., 2017). The block cross-validation increases the spatial independence of the training and testing datasets and evaluates model transferability.

4.3 RESULTS

4.3.1 Aphid spatial patterns and point density distribution

The Average Nearest Neighbour (ANN) distance ratio for *L. e. pseudobrassicae* was *R*= 0.393 and had a z-score of -10.952 which is higher than the critical value of -1.95 at a significance level of 5% with a p-value of 0.000 indicating that the spatial pattern of *L. e. pseudobrassicae* points is clustered and is statistically significant. It also indicates that the spatial pattern did not occur by a random chance (Appendix 5). Similarly, the ANN distance ratio for *M. persicae* was *R* = 0.372 and had a z-score of -10.465 which is also higher than the critical value of -1.95 at a significance level of 5% with a p-value of 0.000 indicating that the spatial pattern of *M. persicae* points is clustered and is statistically significant. It also indicates that the spatial pattern did not occur by a random chance (Appendix 5). The clustered distribution patterns are visualized in Figs. 4.1A and 4.1B for *L. e. pseudobrassicae* and *M. persicae* respectively, following the point density distribution analysis. The red areas on the map indicate points of highest aphid density, yellow areas are intermediate, whilst the blue areas are of the lowest density (Fig 4.1). The maps also show the clustered distribution patterns of both *L. e. pseudobrassicae* and *M. persicae*

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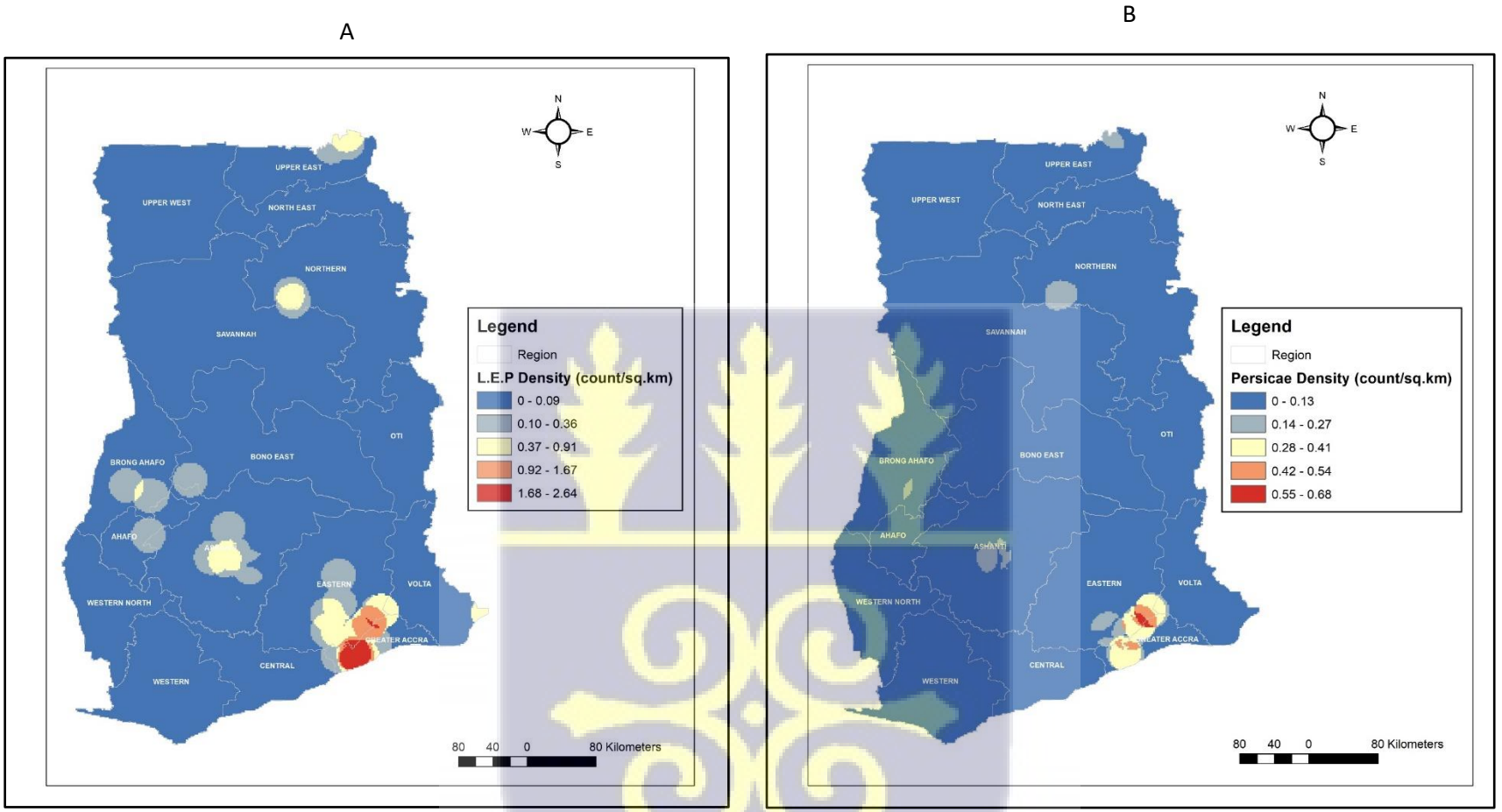


Figure 4-1: Point density distribution of *L. e. pseudobrassicae* (A) and *M. persicae* (B)

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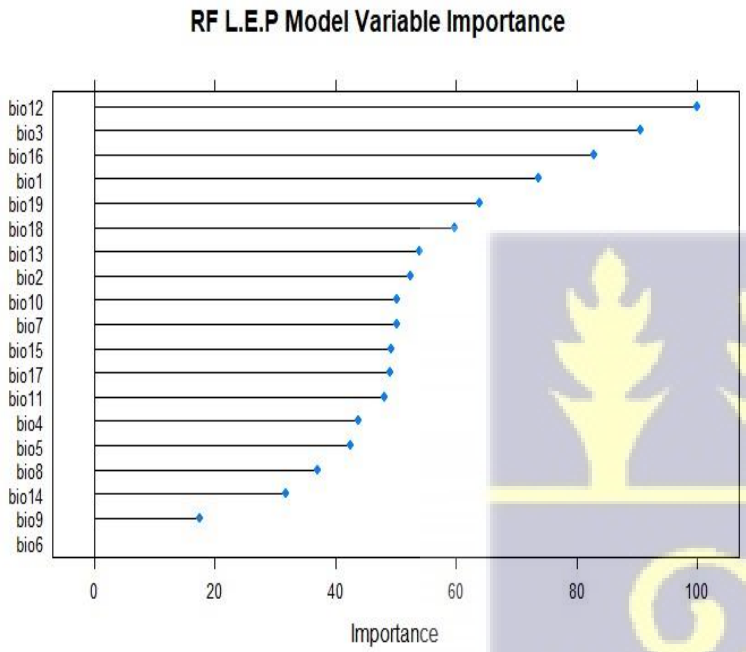
4.3.2 Contributing factors of aphid spatial distribution pattern

The final model for *L. e. pseudobrassicae* had an explained variance of 59.85% whilst that of *M. persicae* was 60.94%. The top 6 variables that explained the variance for *L. e. pseudobrassicae* response variable were BIO12, BIO3, BIO16, BIO1, BIO19 and BIO18 whereas that of *M. persicae* were BIO3, BIO19, BIO1, BIO12, BIO16 and BIO5. It is thus evident that BIO1, BIO3, BIO12, BIO16, BIO19 are strong driving factors for the distribution of both species hence the removal of any of these variables from the model would result in a significant drop in the accuracy. Figures 4.2A and 4.2B show the hierarchy of variable importance in the observed clustered spatial patterns and point densities of *L.e. pseudobrassicae* and *M. persicae,* respectively.

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A



B

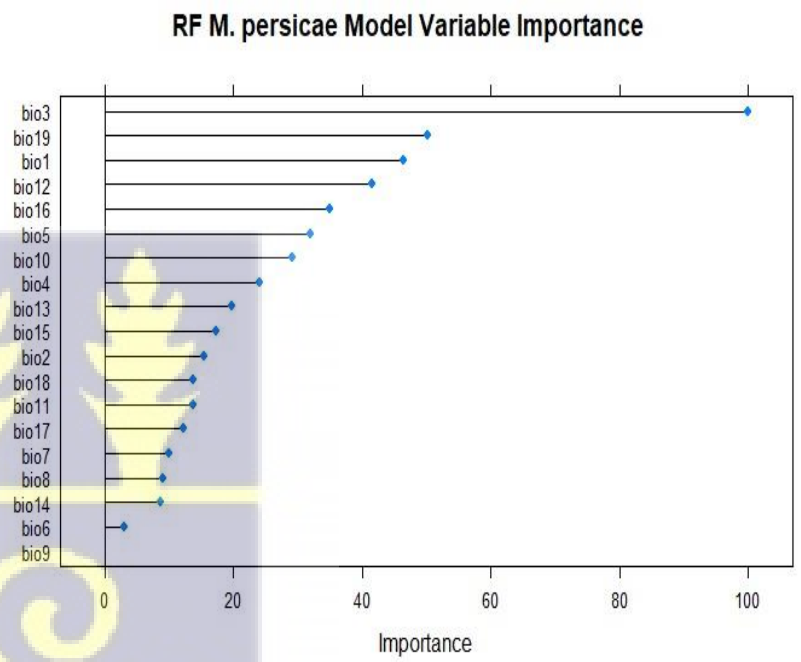


Figure 4-2: Random Forest variable importance plots for (A) *Lipaphis e. pseudobrassicae* and (B) *M. persicae* modelling

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4.3.3 Aphid habitat suitability mapping

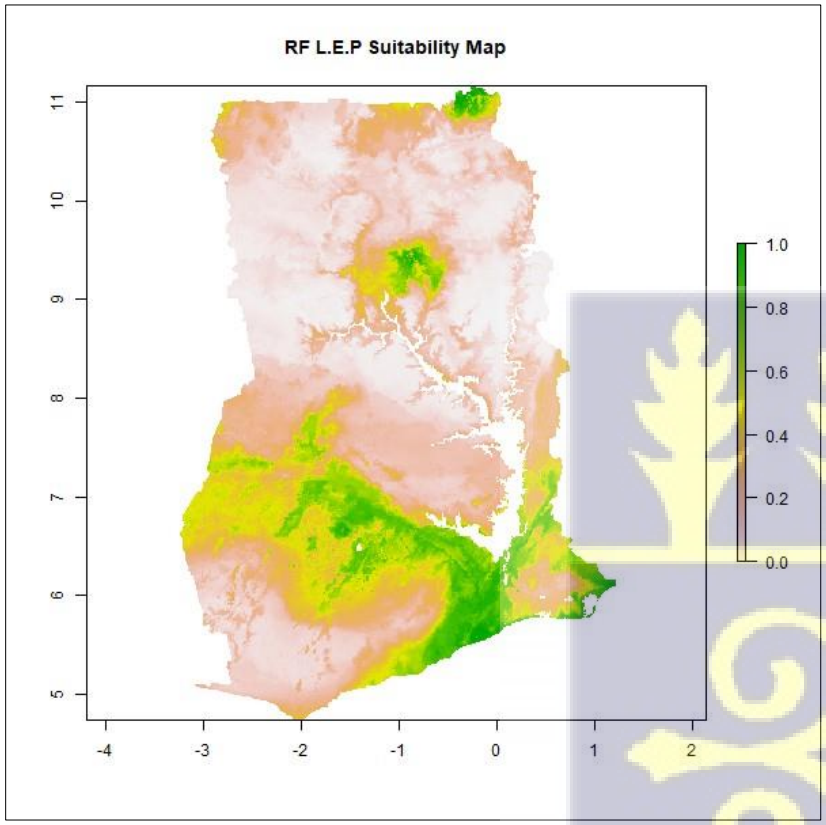
The trained random forest model predicted the probability of occurrence at every pixel location over Ghana, giving a suitability map for *L. e. pseudobrassicae* and *M. persicae* distribution based on the bioclimatic explanatory variables. The colour ramp on the maps begins from 0 and ends at 1, indicating the probability at which a given location would be a suitable habitat for the species. Green areas are of the highest probability, whilst brown areas are of moderate probability and grey areas are of the lowest probability. This is shown in Figs. 4.3 for both aphids.

The predictive model used in this study agreed generally with the occurrence of *L. e. pseudobrassicae* and *M. persicae* in locations that already had a record of infestation. The predicted model is compatible with the real distribution of both aphids in Ghana and shows other areas with a high probability of aphid occurrence such as the Western and Upper West regions. In the Greater Accra region, the potential distribution of *L. e. pseudobrassicae* and *M. persicae* occupies most of the already infested areas. The Upper East, Western, Ashanti, Volta, Greater Accra, and Eastern regions showed a high to very high habitat suitability respectively for both *L. e. pseudobrassicae* and *M. persicae.* The map also shows suitable habitats for both aphids in the Oti region, which had no true records reported for most of the districts except Hohoe, where cabbage cultivation with the infestation of *L. e. pseudobrassicae* was recorded. The northern region shows higher habitat suitability to *M. persicae* compared to *L. e. pseudobrassicae.*

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A



B

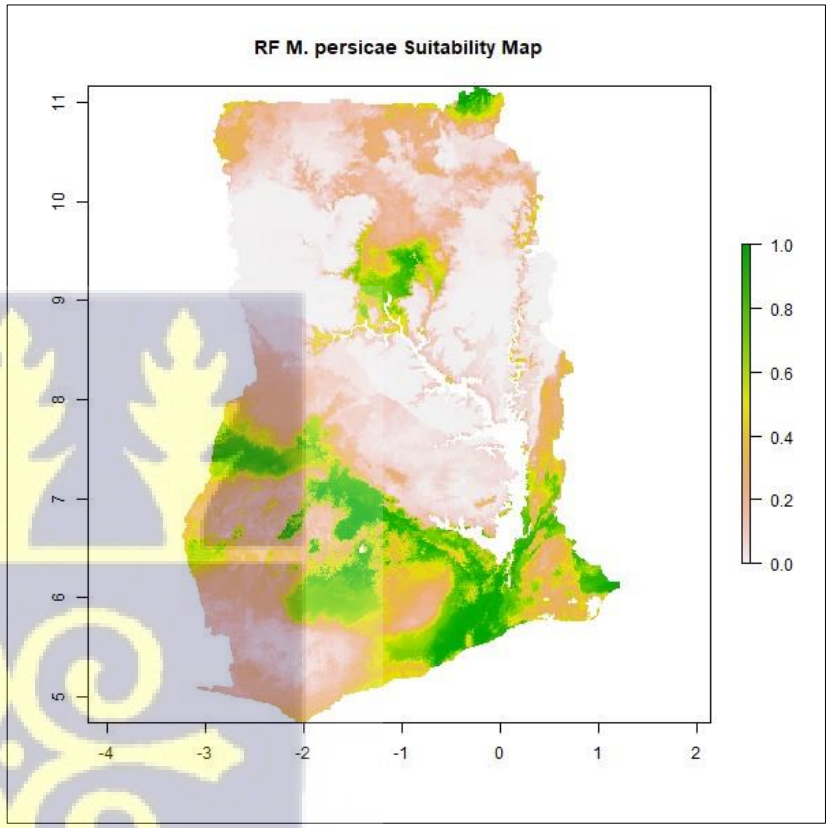


Figure 4-3: Random Forest current habitat suitability map for *Lipaphis erysimi pseudobrassicae* (A) and *Myzus persicae* (B).

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4.4 DISCUSSION

4.4.1 Spatial pattern of *L.e pseudobrassicae* and *M. persicae* and contributing climatic factors

This study showed that both aphids *L.e pseudobrassicae* and *M. persicae* had clustered spatial patterns, suggesting that their occurrence at a given point in time is not by a random chance. These patterns also show spatial heterogeneity where there is an uneven distribution of aphids within a given area. Southwood (1978) and Morris *et al* (1992) reported that most insect populations are spatially aggregated but the degree might differ depending on the insect species. Godfrey and Chaney (1995) recorded clustered spatial distribution patterns of aphid populations on celery. The spatial pattern observed in the distribution of *L.e pseudobrassicae* and *M. persicae* can also be associated with the location of cabbage farms across the country. Cabbage is a popular vegetable, however not indigenous to Ghana, and so not cultivated in the entire country. Additionally, *L. e pseudobrassicae* is a specialist aphid on brassica crops and will only be found on cabbage and related species, accounting for the clustered spatial pattern observed. *M. persicae* on the other hand is a highly polyphagous aphid feeding on several crops, however, during this study, sampling was only done on cabbage, which also accounts for the spatial distribution pattern observed. Notwithstanding, point patterns are only synthetic expressions at a given point in time of processes operating continuously over space (Sandford *et al*., 1978), thus, representing only an event in time and space (Taylor, 1977), and do not provide information about underlying processes supporting the observed patterns (Amedeo and Golledge, 1975). Following multiple contributions, Chen and Shah (2018) further clarified that very few assumptions are made in nearest neighbour methods since they are non-parametric and often allow data to more directly drive predictions.

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Further analysis using species distribution modelling, to understand the underlying contributing factors for the observed spatial pattern showed that BIO12, BIO3, BIO16, BIO1, BIO19, and BIO18 were the underlying contributing factors for the pattern observed in *L.e pseudobrassicae* spatial distribution, and BIO3, BIO19, BIO1, BIO12, BIO16 and BIO5 for *M. persicae.* In combination, BIO1, BIO3, BIO12, BIO16, BIO19 were seen as the major driving factors for the distribution of both species. BIO1 is annual mean temperature, BIO3 is isothermality, BIO12 is annual precipitation, BIO16 is the average precipitation of the wettest quarter, and BIO19 is the average precipitation of the coolest quarter. A study by Yeh *et al*. (2021) emphasized that temperature, isothermality, and precipitation of the coolest quarter (BIO1, BIO3, and BIO19, respectively) are the most significant variables that affect aphid distribution. These provide an understanding of the dynamics of processes that produces a particular observed pattern.

4.4.2 Habitat Suitability of *L.e. pseudobrassicae* and *M. persicae* in Ghana

Lipaphis. e. pseudobrassicae and *M. persicae* are destructive pests of brassica vegetables worldwide and pose a major threat to cabbage production. The current study provides a distribution map of both aphids in Ghana, in relation to changing historical climatic conditions. These maps are relevant to pest management implementation strategies, as they can predict regions suitable for the establishment of these pests outside their actual currently-known distribution. These model maps also reveal the influence of changing climatic factors on the distributions of *L. e. pseudobrassicae* and *M. persicae*, which could be improved upon and adapted for future pest predictions in Ghana

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Previous studies have used machine learning models such as Maxent to map the habitat suitability of other aphid species (Wieczorek and Bugaj-Nawrocka, 2014; Yeh *et al.*, 2021) and these maps were used to inform risk assessment and target surveillance and monitoring efforts towards identifying susceptible areas of pest spread and invasion. Another powerful tool is Random Forest which combines many classification trees to produce more accurate classifications (Cutler *et al*., 2007). Our Random Forest model showed that the predicted habitat suitability firmly agrees 59.85% for *L. e. pseudobrassicae* and 60.94% for *M. persicae* with the actual occurrence of *L. e. pseudobrassicae* and *M. persicae* given the climatic records used, thus suggesting a strong relationship between the model and the ecology of the aphids. This model showed that temperature was the most significant variable that affected the distribution of both aphids.

The current model for the potential distribution of aphids in Ghana corresponds with present-day distributions except for minor changes and non-confirmation for sites that have not yet been visited. Nevertheless, the presence of *L. e. pseudobrassicae* is only restricted to regions where brassica crops are grown since it is a specialist brassica aphid. The model showed more favourable habitats for both *L. e. pseudobrassicae* and *M. persicae* in the South of Ghana, which is already noted as the major hubs for cabbage cultivation.

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CHAPTER 5

5.0 Incidence and seasonal variation of aphids and associated natural enemies on cabbage

5.1 INTRODUCTION

Aphids often pose a major threat to vegetable production because of their rapid development of resistance to available insecticides, thus, the need for an integrated approach. Developing integrated approaches in pest management often requires a thorough understanding of the pest biology and ecology, that is its interaction with the environment which is made up of biotic and abiotic factors that regulate pest populations (Dent and Blinks, 2020). The abundance of aphids on crops is mostly influenced by the host plant quality, climate, inter-specific and intra-specific interactions, and host plant density (Van Emden and Bashford 1971; Agarwala and Datta, 1999). Biotic factors such as natural enemies, competing organisms, and abiotic factors such as rainfall, temperature, and relative humidity, can affect the population growth rate and density of aphids.

Understanding the dynamics of pests, as well as their association with the environment, allows for critical sampling periods and the adoption and successful implementation of appropriate management strategies. Implementing a timely management strategy prevents pest build-up beyond economic thresholds and minimizes the over usage of insecticides (Barzman *et al*., 2015; Dara, 2019).

This study therefore aimed at understanding the incidence and seasonal variation of *L. e pseudobrassicae* and *M. persicae* (the two most abundant species) in relation to climatic factors, as well as their associated natural enemies.

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5.2 MATERIALS AND METHODS

5.2.1 Study sites

This study was carried out in two locations; the University of Ghana Soil and Irrigation Research Center (SIREC), Kpong (Lat. 6.134874, long. 0.08207) and a farmer's farmland at Aseseeso (Lat. 6.009533, Long. -0.0443213), in the Eastern region of Ghana, in the Coastal Savanna and Semi-Deciduous Forest agroecological zones respectively. These two agroecological zones were selected based on results obtained in Chapter 3; that more cabbage was grown in the south especially in the Coastal Savannah (CS) and Deciduous Forest (DF) zones. Kpong is characterized by an annual rainfall between 700 and 1100mm, an average annual temperature of 28 ˚C, and relative humidity between 59%-93% while Asseseeso is characterized by an annual rainfall between 900 and 1270mm, an average annual temperature of 25˚C, and relative humidity between 72%-95% (GHWC-data, 2021). Both locations have two seasons with a bimodal rainfall pattern which includes a major rainy season (April to July), and a minor rainy season (August to November), and a dry season (December to March) (Ghana Meteorological Center).

5.2.2 Nursery establishment

Certified hybrid white cabbage (*B. oleracea* var. capitata) (cv. Oxylus) seeds were purchased from AGRI-SEED Limited, Accra, Ghana. The oxylus variety was used because it is the most widely grown cabbage variety in Ghana (Timbilla and Nyarko, 2004). The seeds were nursed in trays (1m X 1m) (Plate 5.1) containing a mixture of soil and decomposed poultry manure (20t/ha). Trays were kept in a screen house at SIREC, Kpong, to prevent infestation by insect pests. Thinning, and irrigation was done regularly as need be as recommended by Oscar (2018).

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Plate 5.1: Nursery in screen house (Kpong).

5.2.3 Land preparation and experimental layout

Land for experimental plots was cleared, ploughed, and ridged one week after nursery establishment. The soil was mixed with decomposed poultry manure at a recommended application rate of 20t/ha (Ogedegbe and Law-Ogbomo, 2013) and allowed for one week before the transplanting of mature seedlings. The experiment was set out in 6 experimental plots, with each plot measuring 3 m x 3m and an inter-plot distance of 0.5 m.

5.2.4 Transplanting

Seedlings were transplanted four weeks after germination when 4-6 true leaves were present at a planting distance of 75cm × 50cm onto 3m x 3m plots giving a total of 35 plants per plot, in 5 rows and 7 columns. No insecticide was applied throughout the study period.

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5.2.5 Study period

The study was carried out for five cropping seasons from 22 August 2019 to 2 February2021 in the Coastal Savannah agroecological zone and from 22 July 2019 to 10 February 2021 in the Deciduous Forest agroecological zone. The seasons have been abbreviated S1 to S5 to denote the cropping seasons one to five. Data on insect abundance were collected weekly, two weeks after transplanting for 10 weeks for S1 to S5. For each season, data collected included the weekly trend of aphid populations on cabbage plants and their associated natural enemies.

Table 5.1: Sampling periods for incidence and seasonal variation of aphids and their natural enemies.

Abbreviation	Season	Sampling period		Seasons
		Coastal Savannah	Deciduous Forest	
S1	Cropping season 1	22 Aug. to 24 Oct. 2019	22 Jul. to 23 Sept. 2019	Minor rainy season
S2	Cropping season 2	20 Nov. 2019 to 14 Jan 2020	12 Nov. 2019 to 7 Jan.2020	Minor rainy to dry season
S3	Cropping season 3	19 Mar. to 21 May	11 Mar. to 7 Jun.	Major rainy season
S4	Cropping season 4	1 Sept. to 2 Nov.	29 Sept. to 30 Nov.	Minor rainy season
S5	Cropping season 5	1 Dec. 2020 to 2 Feb. 2021	9 Dec. 2020 to 10 Feb. 2021	Dry season

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5.2.6 Sampling for aphids and their natural enemies

The population density of *L. e. pseudobrassicae, M. persicae* and their associated natural enemies was evaluated at 7-day intervals, from 5 to 6 true-leaf stage (two weeks after transplanting) to the head fill stage (Andalaro *et al*., 1983). Sampling for aphids was modified from the method according to Hughes (1963) and Fidelis e*t al*. (2019), which involved destructive sampling of leaves of cabbage plants. During each sampling, the third, fourth and fifth expanded leaves of 20 randomly selected cabbage plants (4 per row) were sampled. Infestation by *L. e. pseudobrassicae* and *M. persicae* were determined by visually counting the number of aphids; apterous, alate, and mummified forms on the underside of the randomly sampled leaves. The other associated natural enemies were also counted and recorded.

Parasitoid population was determined by counting the number of parasitized aphids (mummies; seen as golden-shiny aphids) on cabbages. Mummified aphids were collected and placed in 100ml plastic pots covered with a mesh, for the emergence of parasitoid adults in the laboratory. Specimens of insect predators and emerged parasitoids were collected into vials using forceps and maintained in 70% alcohol for further identification. Data on parasitized aphids were used to calculate the percentage parasitism of aphids.

5.2.7 Meteorological data collection

Meteorological data which includes average daily temperature, rainfall and percentage relative humidity was obtained from the University of Ghana Soil and Irrigation Research Centre weather station, Kpong and from the National Ghana Meteorological Agency, Accra for the period 2019

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to 2021. Weekly averages were calculated from the data and used in the correlation and regression analyses.

5.2.8 Insect identification.

Visual field identification of natural enemies was done following the aphid natural enemy field guide by Gardiner *et al*. (2012) and the guide to the identification of aphids and their natural enemies by Campos and García-Marí (2014). Identification of syrphid flies, parasitoids and hyperparasitoids were further confirmed following the molecular identification procedure outlined in Section 3.1.3.1. Identification of other natural enemies was confirmed by a taxonomist (Mr. Davis) at the Plant Protection and Regulatory Service Department (PPRSD) of the Ministry of Food and Agriculture (MoFA).

5.2.9 Parasitism of aphids

The percentage parasitism of aphid species (% P) was calculated using Formula 5.1 and the mean percentage parasitism was determined for all seasons in both agroecological zones.

$$\%Parasitism = \frac{No.\ of\ parasitized\ individuals\ of\ species}{Total\ No.\ of\ individual\ collected}\ X\ 100 \qquad \text{(Formula 5.1)}$$

5.2.10 Data analysis

Percentage data were transformed using the logit function, and Shapiro's test for normality was carried out. Transformed data were normal, hence subjected to ANOVA at $\alpha = 0.05$. Mean separation was done using Student Neuman Keul's test (SNK) at 5% significance level. Shapiro's test for normality and Levene's test for variance homogeneity were conducted on insect weekly count data, and the data were not normally distributed/homogenous. The data were therefore

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square root transformed, and further tested for normality and homogeneity. Data were normal, and subjected to repeated measurements of ANOVA at a 5% significance level to determine the population variation across seasons. An independent student t-test was used to determine the difference between aphid and natural enemy populations between the two agroecological zones.To understand the relationship between populations of aphids (dependent variables) and weather parameters/natural enemies (explanatory variables), a fixed effect generalized linear model with a negative binomial ang log-link function was adopted, to account for overdispersion. All analysis were carried out in SPSS V22.

5.3 RESULTS

5.3.1 Abundance of aphids

Lipaphis erysimi pseudobrassicae, was more abundant than *Myzus persicae* with a relative abundance of 90.4% and 9.6%, respectively in the Coastal Savannah agroecological zone and 68.1% and 31.9%, respectively in the Deciduous Forest agroecological zone. The densities of *M. persicae* varied significantly between the two agroecological zones ($t(59) = 2.39$, $p = 0.020$).

5.3.2 Diversity and abundance of aphid natural enemies

5.3.2.1 Abundance of aphid natural enemies

In total five predator species were identified in the Coastal Savannah agroecological zone: Hoverflies; *Paragus borbonicus* (Macquart) (Diptera: Syrphidae), Ladybird beetles; *Cheilomenes* spp (Coleoptera: Coccinelidae), aphid midge; *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae), *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), and spiders) and one

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parasitoid: *Aphelinus varipes* (Förster) (Hymenoptera: Aphelinidae). In the Deciduous Forest agroecological zone, six predator species were identified: *Paederus* sp. (Coleoptera: Staphyllinidae), *Paragus borbonicus* (Macquart), *Cheilomenes* spp., *Aphidoletes aphidimyza* (Rondani), *Chrysoperla carnea* (Stephens), and spiders, one parasitoid: *Aphelinus varipes* (Förster) and one hyperparasitoid: *Alloxysta brevis* (Thomson) (Hymenoptera: Charipidae) (Table 5.2). The abundance of these natural enemies differed between cropping seasons and across the two agroecological zones. The most abundant natural enemies were spiders (Araneae) and insect species belonging to the order Diptera, followed by Neuroptera and Coleoptera (Table 5.2).

The hoverfly *Paragus borbonicus* and spiders were consistently recorded in all the cropping seasons (S1-S5) in both agroecological zones compared to the other natural enemies. *Paederus* sp. was recorded only in the last two cropping seasons (S4 and S5) in the Deciduous Forest agroecological zone. The hyperparasitoid *Alloxysta brevis* was recorded in the first, fourth and fifth cropping seasons (S1, S4 and S5 respectively) only in the Deciduous Forest agroecological zone. An independent t-test showed significant differences in the density of *Paederus* sp ($t(93) = -3.00$, $p = 0.003$) and *Alloxysta brevis* ($t(93) = 2.46$, $p = 0.016$) between the two agroecological zones.

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Table 5.2: Abundance of aphid natural enemies in the CS and DF AEZs across five cropping seasons

Group	Species	Family	Coastal Savannah zone		Deciduous Forest zone		t-test
			Tot.	Abundance (%)	Tot.	Abundance (%)	
Predators							
Diptera	***Paragus borbonicus***	Syrphidae	757	42.26	745	30.84	0.985
	Aphidoletes aphidimyza	Cecidomyiidae	100	5.58	200	8.28	0.261
Coleoptera	***Cheilomenes* sp.**	Coccinellidae	125	6.97	83	3.43	0.425
	***Paederus* sp.**	Staphylinidae	0	0	32	1.32	0.003
Neuroptera	***Chrysoperla carnea***	Chrysopidae	90	5.02	165	6.83	0.186
	Spiders	Araneae	669	37.35	673	27.86	0.781
Parasitoids							
Hymenoptera	***Aphelinus varipes***	Braconidae	50	2.79	316	13.08	0.158
	Alloxysta brevis	Charipidae	0	0	201	8.32	0.016
Total			1791		2415		

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5.3.2.2 Shannon Weiner diversity indices and Shannon Evenness for natural enemies of aphids across five cropping seasons in two AEZs

Table 5.3 below shows the Shannon-diversity indices (H') and Shannon Effective Number of aphid natural enemies for five cropping seasons in the CS and DF agroecological zones. From the study, the highest diversity indices were recorded in season 1 for both the CS and DF agroecological zones (H'=1.56 and H'=1.6 respectively) with a corresponding high Shannon Evenness (Table 12).

In the coastal savanna AEZ, the first season had the highest aphid natural enemy diversity (H'=1.56) while the least diversity was recorded in the third season (H'=0.6). The fourth and the fifth seasons had the same aphid natural enemy diversity indices (H' = 0.7). In the Deciduous Forest AEZ, the highest aphid natural enemy diversity index was recorded in the first season (H'=1.6), followed by the third and fifth seasons, with the fourth season recording the least diversity, with a diversity index of (H'=0.6) (Table 5.3). Generally, more aphid natural enemy diversity was recorded in the Deciduous Forest AEZ compared to the Coastal Savanna AEZ in all cropping seasons (Table 5.3).

Table 5.3 Shannon Weiner diversity indices and Evenness for aphid natural enemies

Insects	Shannon Weiner Diversity Indices (H')									
	Coastal Savannah AEZ					Deciduous Forest AEZ				
	S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
Natural enemies	1.56	0.9	0.6	0.7	0.7	1.6	0.7	0.9	0.6	0.9
	Shannon Evenness									
Natural enemies	1.73	0.95	0.69	0.69	0.79					0.4

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5.3.3 Seasonal incidence and population fluctuation of aphids on cabbage

5.3.3.1 *Lipaphis erysimi pseudobrassicae* (Davis)

In the Deciduous Forest agroecological zone, all the seasons had different variating trends for the population dynamics of *L. e. pseudobrassicae*. In S1, S2 and S5, the incidence of *L. e. pseudobrassicae* on cabbage commenced during the pre-vegetative stage (2 weeks after transplanting), while in S4, incidence commenced during the early vegetative stage (3-4 weeks after transplanting), and during early head formation stage (7-8 weeks after transplanting) in S3. The highest population peaks were recorded in S4 during the cupping stage (5-6 weeks after transplanting), followed by S1 and S2 during the early vegetative stages. The lowest population density was recorded in S2 and S3, albeit with population peaks during the early head formation stage (Fig. 5.1A). There was a significant difference in *L. e. pseudobrassicae* population incidence across the different cropping seasons, and between cabbage growth stages ($F_{4, 20} = 9.57$; $P < .001$ and $F_{9,215} = 29.64$; $P < .001$, respectively). A significant interaction between the seasons and cabbage growth stages (weeks) was also recorded for the Deciduous Forest zone ($F_{34, 215} = 21.54$; $P < .001$).

In the Coastal Savannah agroecological zone, *L. e. pseudobrassicae* incidence was first recorded during the early vegetative stage in S1, S2 and S4, the cupping stage in S5 and the head formation stage in S3. The highest peaks were recorded during the early head formation stage in S2, followed by S4, with peaks during the early vegetative stage and the cupping stage(Fig. 5.1B). Significant difference was recorded in *L. e. pseudobrassicae* population incidence across the different seasons, and between plant growth stages ($F_{4, 20} = 57.36$; $P < .001$ and $F_{9, 220} = 44.s17$; $P < .001$, respectively). There was a significant interaction between the seasons and cabbage growth stages ($F_{35,220} = 19.77$; $P < .001$).

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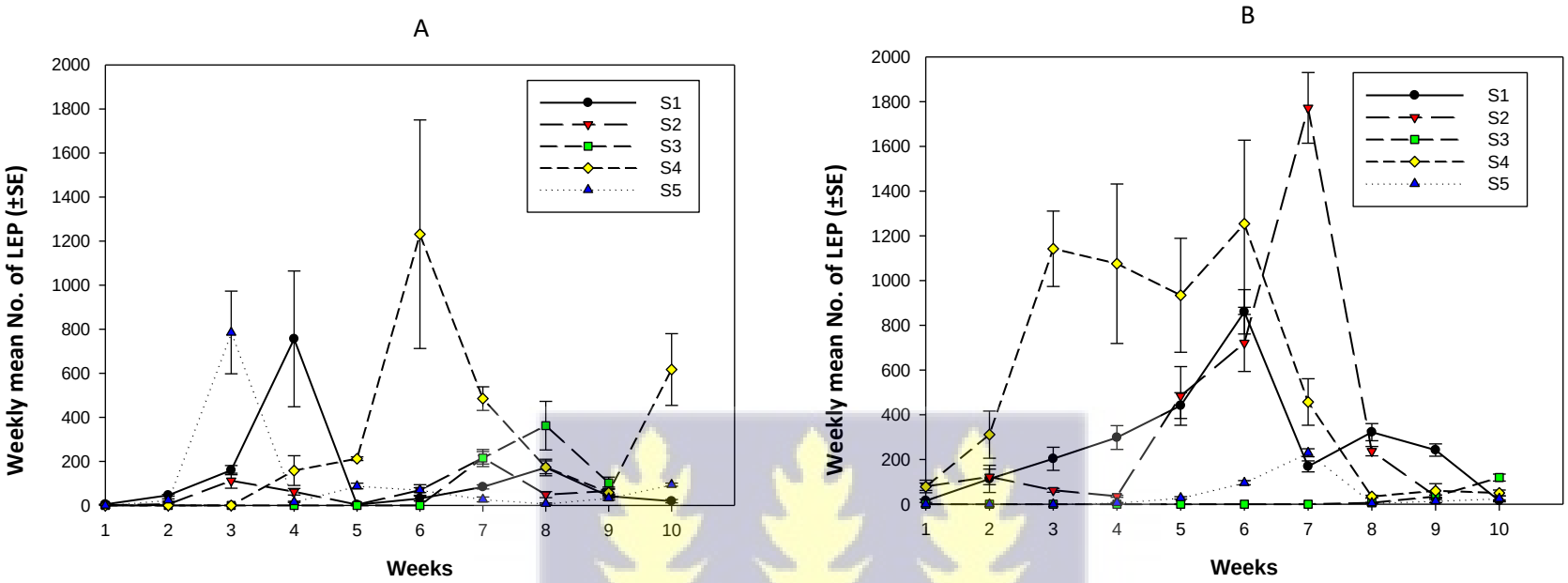


Figure 5-1: Seasonal incidence and population fluctuation of *Lipaphis erysimi pseudobrassicae* (LEP) (±SE) in the Deciduous Forest (A) and Coastal Savannah (B) agroecological zones across five cropping seasons (2019-2021).

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5.3.3.2 *Myzus persicae* (Sulzer)

Fig. 5.2 shows the seasonal population fluctuations of *M. persicae* in the Deciduous Forest and Coastal Savannah agroecological zones of Ghana. The density of this species ranged from 0 to 500 aphids per leaf for the entire sampling season. In the Deciduous Forest agroecological zone, the incidence was recorded from the pre-vegetative stage in S1 and S5, early vegetative stage in S2, early head formation stage in S3 and cupping stage in S4. The Highest *M. persicae* population density was recorded in S1, with a steady increase and a sharp peak during the early vegetative stage. The lowest populations were recorded in S2 and S3 (Fig. 5.2A). There was a significant difference in *M. persicae* population incidence across the different seasons, and between the plant growth stages ($F_{4, 20} = 35.89$; $P < .001$ and $F_{9,215} = 57.65$; $P < .001$, respectively). A significant interaction between the seasons and cabbage growth stages was also recorded for the Deciduous Forest agroecological zone ($F_{34,215} = 17.89$; $P < .001$).

In the Coastal Savannah agroecological zone, *M. persicae* incidence was recorded in the pre-vegetative stage in S1, S2 and S4, and in the cupping stage in S5. No aphids were recorded in S3 (Fig. 5.2B). The population fluctuation differed for all seasons, with peak population attained during the early vegetative and cupping stage in S3. In S1, peak population densities were recorded during the cupping and early head formation stages. There was a significant difference in *M. persicae* population incidence across the different seasons, and between the plant growth stages ($F_{4, 20} = 63.67$; $P < .001$ and $F_{9, 220} = 54.39$; $P < .001$, respectively). A significant interaction between the seasons and growth stages was also recorded for the Coastal Savannah agroecological zone ($F_{35,220} = 12.03$; $P < .001$).

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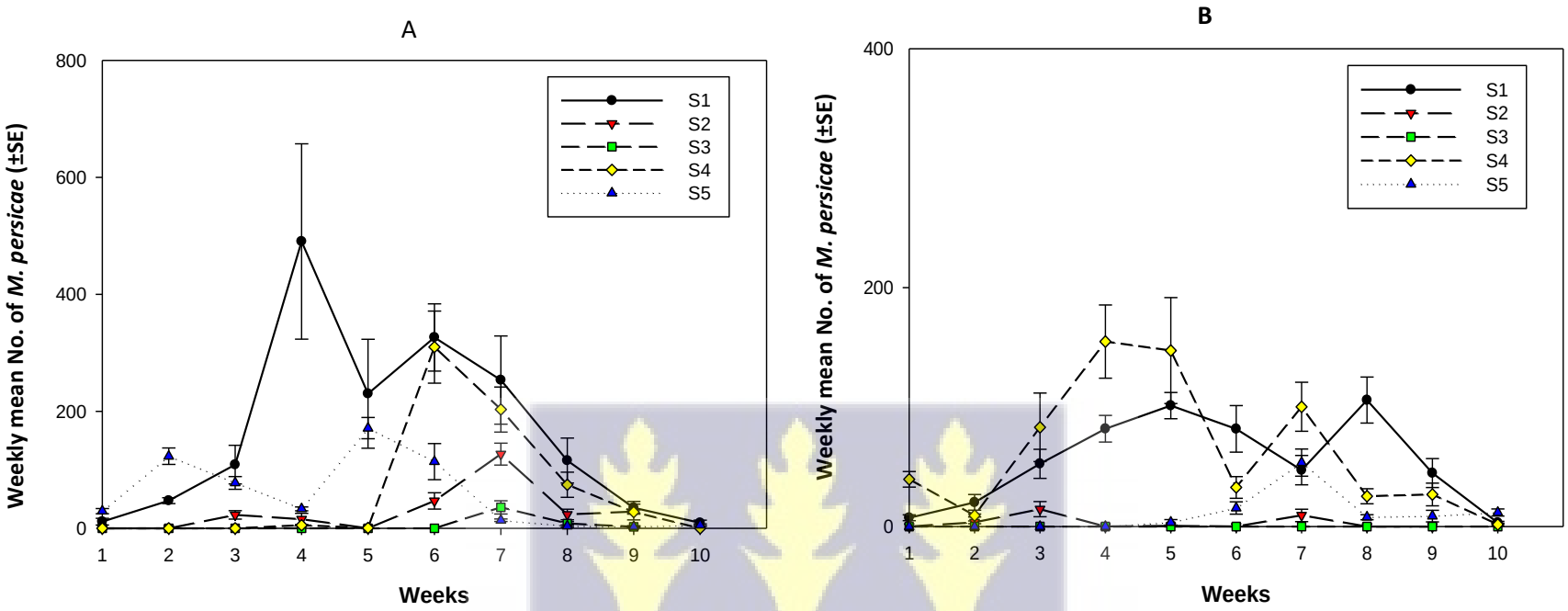


Figure 5-2: Seasonal incidence and population fluctuations of *M. persicae* (±SE) in the Deciduous Forest (A) and Coastal Savannah (B) agroecological zones across five cropping seasons (2019-2021).

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5.3.4 Seasonal incidence and population fluctuations of aphids' natural enemies

5.3.4.1 *Paragus borbonicus*

The occurrence and seasonal population fluctuations of *P. borbonicus* is shown in Fig. 5.3, and it varied between the Deciduous Forest and Coastal Savannah agroecological zones of Ghana. In the Deciduous Forest agroecological zone, the incidence of *P. borbonicus* was first recorded during the pre-vegetative stage in S1, the early vegetative stage in S2 and S5, the cupping stage in S4, and the early head formation stage in S3. The population steadily increased with different seasonal fluctuations, and the highest peaks were attained during the early vegetative stage in S1, and the cupping stage in S4. The lowest population density was recorded in S2, with average weekly mean density oscillating between 0 to 5 (±SE) (Fig. 5.3A). There was a significant difference in *P. borbonicus* population incidence between the different seasons, and between the plant growth stages ($F_{4, 20} = 14.01$; $P < .001$ and $F_{9,215} = 33.49$; $P < .001$, respectively). A significant interaction between the seasons and growth stages was also recorded in this agroecological zone ($F_{34,215} = 21.10$; $P < .001$).

In the Coastal Savannah agroecological zone, the incidence of *P. borbonicus* was recorded first during the pre-vegetative stage in S2 and S4, early vegetative stage in S1, cupping stage in S5 and head formation stage for S3. The highest peak was recorded in S2 and S5, during the early head formation stage, and during the cupping stage in S1 and S4. S3 had the lowest *P. borbonicus* population density and was only recorded during the head formation stage (Fig. 5.3B). There was a significant difference in *P. borbonicus* population incidence between seasons, and between plant growth stages ($F_{4, 20} = 25.10$; $P < .001$ and $F_{9, 220} = 62.51$; $P < .001$, respectively). A significant interaction between the seasons and growth stages was also recorded for this agroecological zone ($F_{35,220} = 18.42$; $P < .001$).

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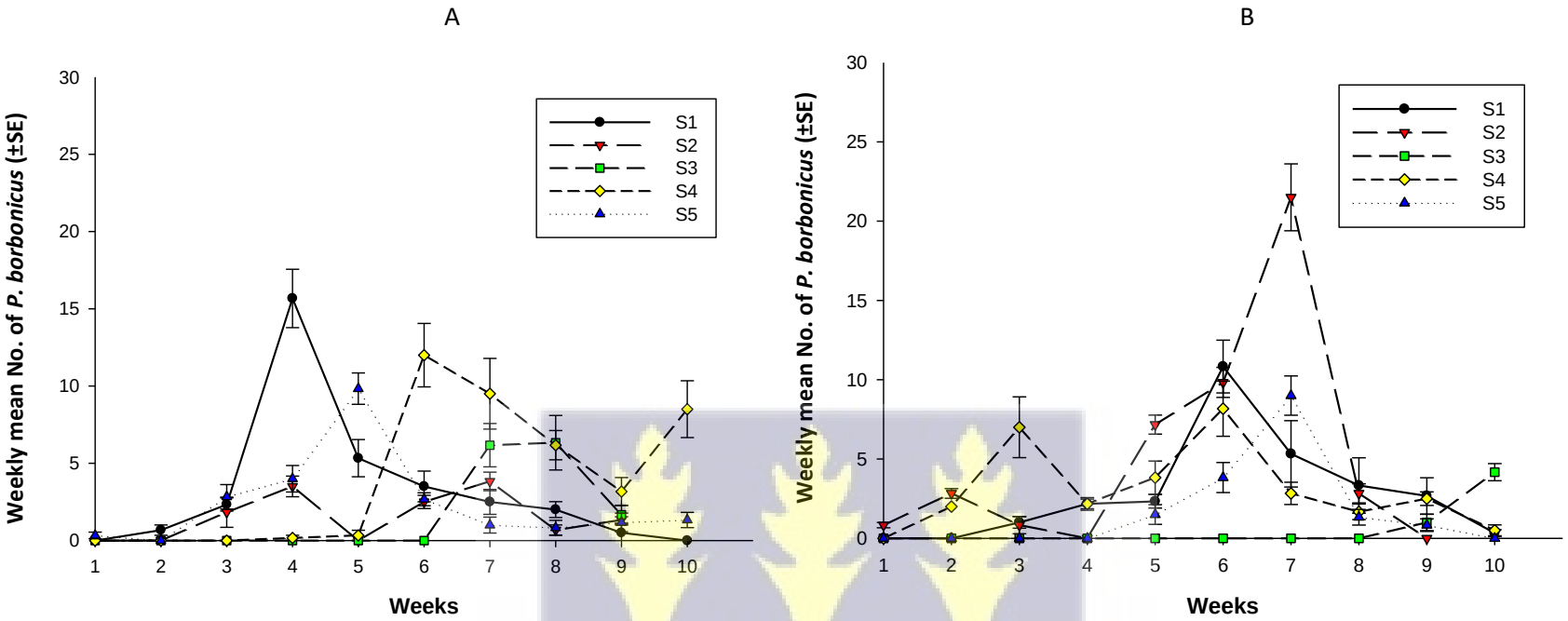


Figure 5-3: Seasonal incidence and population fluctuations of the hoverfly *Paragus borbonicus* (±SE) in the Deciduous Forest (A) and Coastal Savannah (B) agroecological zones across five cropping seasons (2019-2021).

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5.3.4.2 *Cheilomenes* spp.

The population density of *Cheilomenes* spp was generally low throughout the five cropping seasons and was only recorded in S1, S2 and S5 in the Deciduous Forest agroecological zone, and in S1, S2, S4 and S5 in the Coastal Savannah agroecological zone. Fig. 5.4 shows the variation of this species in the Deciduous Forest and Coastal Savannah agroecological zones. In the Deciduous Forest zone, records of *Cheilomenes* spp occurrence were observed first during the early vegetative stage in all cropping seasons (S1, S2 and S5), with population peaks during the early vegetative and early head formation stages in S1 (Fig. 5.4A). Nevertheless, significant differences were recorded between seasons and plant growth stages ($F_{4, 20} = 74.31$; $P < .001$ and $F_{9,215} = 23.42$; $P < .001$ respectively), with a significant interaction between seasons and growth stages ($F_{34,215} = 16.41$; $P < .001$).

The incidence of *Cheilomenes* spp in the Coastal Savannah agroecological zone was observed during the early vegetative stage in S1 and S4, pre-vegetative stage in S2 and early head formation stage in S3 and S5. The highest peak population density was recorded during the cupping stage in S1 (Fig. 5.4B). Although incidence was low in all the seasons in the Coastal Savannah zone, significant differences were recorded between seasons and plant growth stages ($F_{4, 20} = 24.31$; $P < .001$ and $F_{9,220} = 10.27$; $P < .001$ respectively), with a significant season: plant growth stage interaction ($F_{35,220} = 9.60$; $P < .001$).

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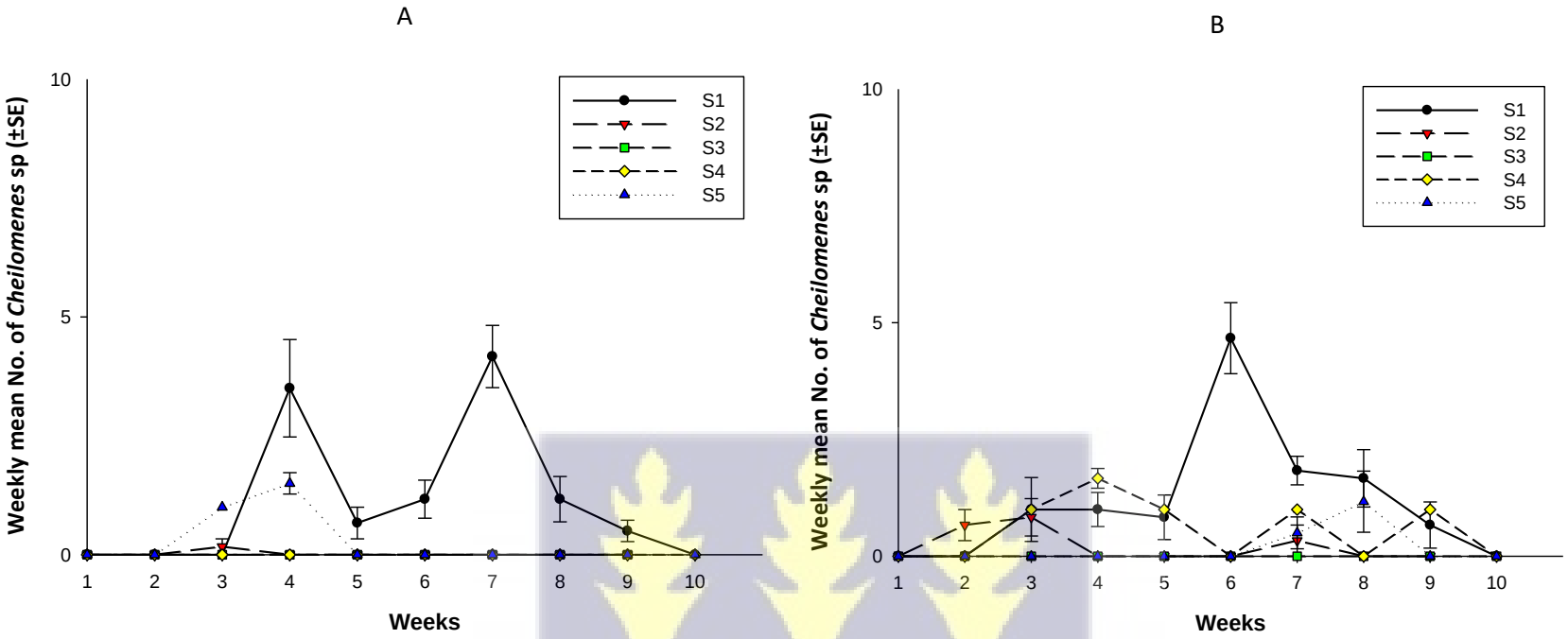


Figure 5-4: Seasonal incidence and population fluctuations of *Cheilomenes spp* (±SE) in the Deciduous Forest (A) and Coastal Savannah (B) agroecological zones across five cropping seasons (2019-2021).

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5.3.4.3 *Chrysoperla carnea*

Fig. 5.5 shows the seasonal variation in the population of *Chrysoperla carnea* on cabbage in the Deciduous Forest and the Coastal Savannah agroecological zones. Like the *Cheilomenes* spp trend, the average population of *C. carnea* was small throughout the study period, with records made only in S1, S3 and S5 in the Deciduous Forest agroecological zone and S1, S2, S4 and S5 in the Coastal Savannah agroecological zone.

In the Deciduous Forest, it first occurred during the early vegetative stage in S1 and S5, and during the early head formation stage in S3. Peak incidence was recorded during the cupping stage in S1 (Fig. 5.5A), nevertheless, there was a significant variation in *C. carnea* population incidence between the five cropping seasons ($F_{4, 20} = 43.93$; $P < .001$), and a significant difference between the different plant growth stages ($F_{9,215} = 12.07$; $P < .001$). Interaction between the seasons and growth stages was also significant ($F_{34,215} = 17.78$; $P < .001$).

In the Coastal Savannah agroecological zone, *C. carnea* incidence was first recorded during the cupping stage in S1, the pre-vegetative stage in S2, and the early head formation stage in S4 and S5, with the highest peak population density during the cupping stage in S1 (Fig. 5.5B). There was a significant variation in *C. carnea* population incidence between the five cropping seasons ($F_{4, 20} = 4.94$; $P = 0.006$), and a significant difference between the plant growth stages ($F_{9,220} = 8.63$; $P < .001$). Interaction between the seasons and plant growth stage was also significant in this agroecological zone ($F_{35,220} = 7.34$; $P < .001$).

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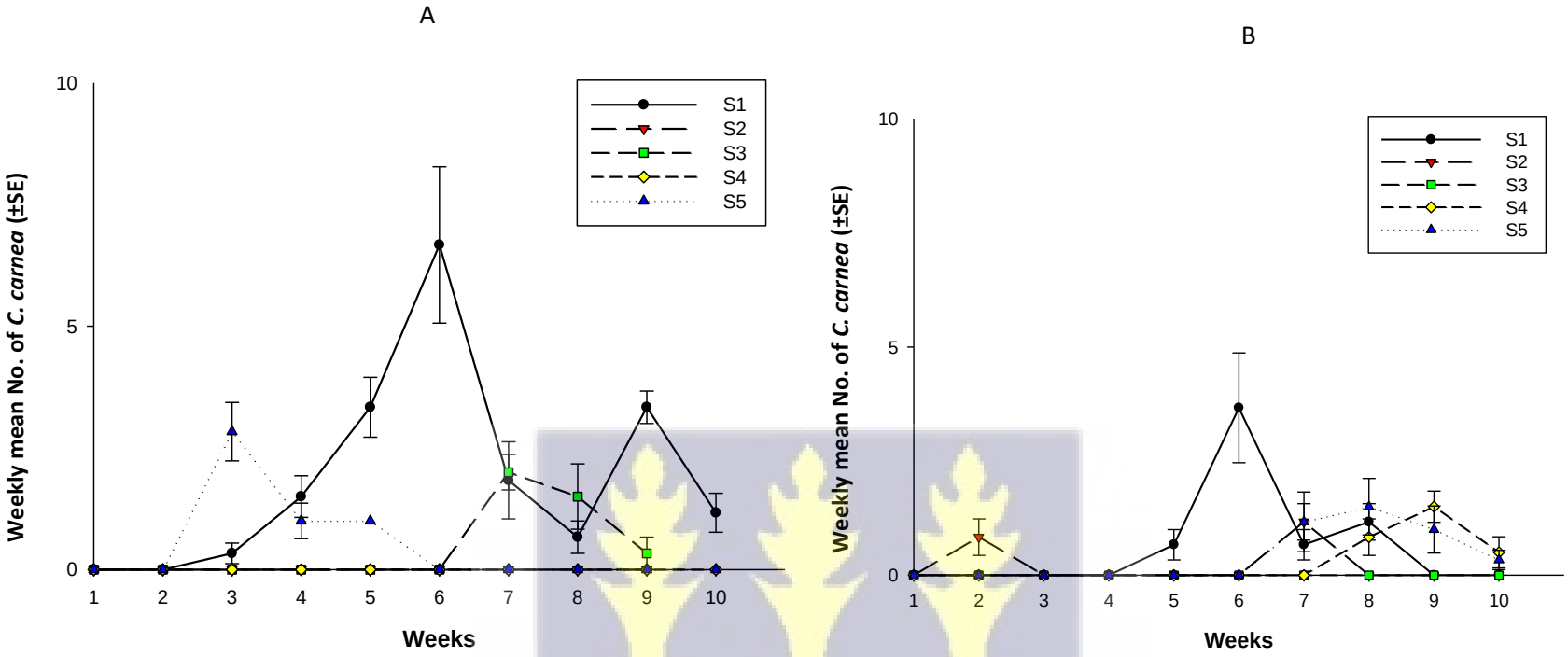


Figure 5-5: Seasonal incidence and population fluctuations of *Chrysoperla carnea* (±SE) in the Deciduous Forest (A) and Coastal Savannah (B) agroecological zones across five cropping seasons (2019-2021).

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5.3.4.4 *Aphidoletes aphidimyza*

In the Deciduous Forest zone, *A. aphidimyza* was only recorded in S3, S4 and S5, while in the Coastal Savannah zone it was recorded in S1 and S3. The incidence of *A. aphidimyza* in the Deciduous Forest was observed during the early head formation stage in S3 and S4, and in the early vegetative stage in S5. The highest population peaks were recorded during cupping in S5 and early head formation in S3 and S4 (Fig. 5.6A). There was a significant variation in *A. aphidimyza* population incidence between seasons ($F_{4, 20} = 61.63$; $P < .001$), and plant growth stages ($F_{9, 215} = 49.20$; $P < .001$), with significant interaction between seasons and plant growth stage ($F_{34, 215} = 32.11$; $P < .001$).

In the Coastal Savannah agroecological zone, the incidence was first recorded during early head formation in S1, and during cupping in S4 with population peaks observed during the early head formation and cupping stages for S1 and S4 respectively (Fig. 5.6B). Significant variation was recorded in *A. aphidimyza* population incidence between seasons ($F_{4, 20} = 13.93$; $P = 0.001$), and plant growth stages ($F_{9, 220} = 11.63$; $P < .001$). Interaction between the seasons and growth stages was also significant ($F_{35, 220} = 13.78$; $P < .001$).

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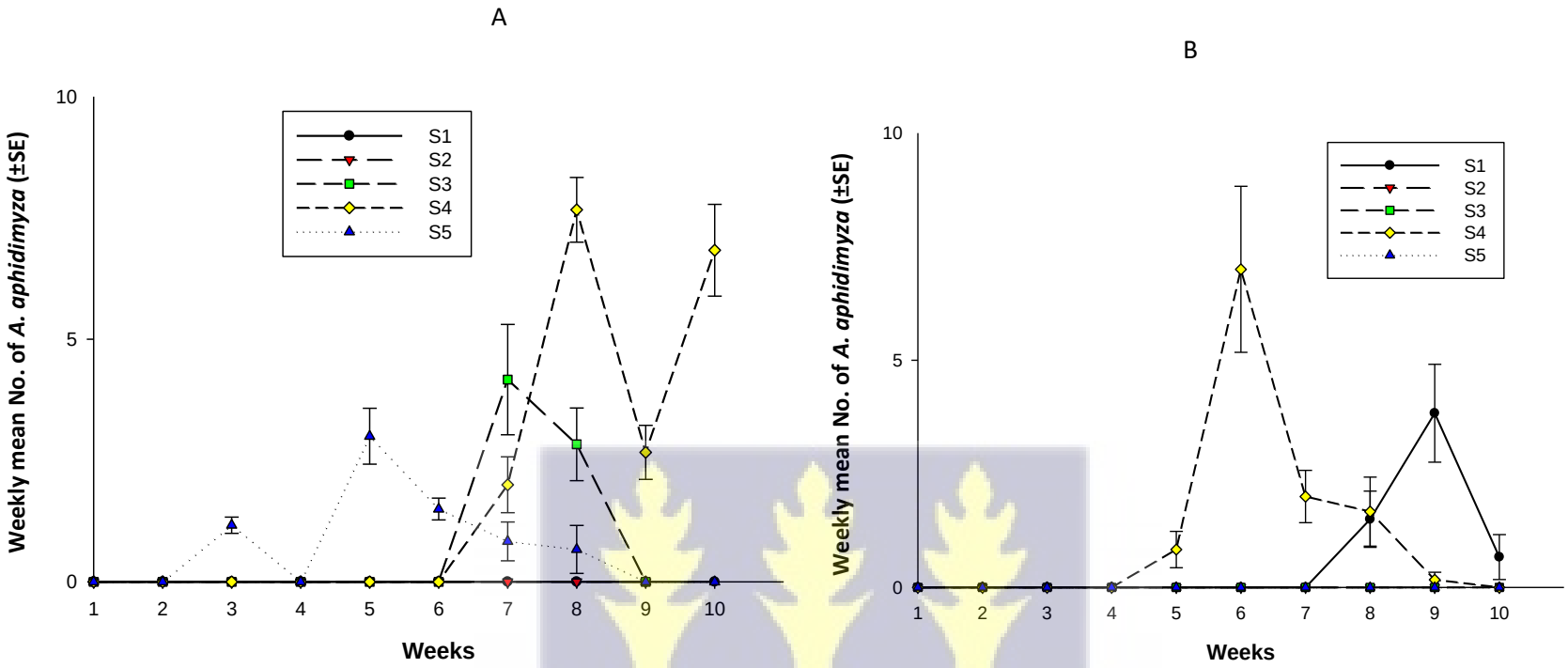


Figure 5-6: Seasonal incidence and variation in the population of *Aphidoletes aphidimyza* (±SE) in the Deciduous Forest (A) and Coastal Savannah (B) agroecological zones across five cropping seasons (2019-2021).

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5.3.4.5 *Aphelinus varipes*

Aphelinus varipes incidence was recorded in S1 and S3 of the Deciduous Forest agroecological zone, and S1 of the Coastal Savannah agroecological zone. No significant differences were recorded between seasons and plant growth stages in the Deciduous Forest agroecological zone; however, the first incidence was recorded during the early vegetative stage of cabbage in both cropping seasons, with a peak population during cupping in S1. In the Coastal Savannah zone, *A. varipes* was only recorded during cupping stage in S1. This is the first report of *A. varipes* as an aphid parasitoid on cabbage in Ghana.

5.3.4.6 *Paederus* sp.

The rove beetle *Paederus* sp. was recorded during the study in Deciduous Forest agroecological zone in S4 and S5, during early head formation and head formation stages. No significant differences were recorded between the seasons and across sampling weeks. However peak populations were recorded during weeks 7 and 9 of S4 and during weeks 8 and 9 of S4 (Head formation stage).

5.3.4.7 Spiders

Fig. 5.7 shows the seasonal population fluctuations of spiders, in the Deciduous Forest and Coastal Savannah agroecological zones of Ghana. The population fluctuation across the five cropping seasons in the Deciduous Forest zone was similar, with peaks recorded during the early vegetative, cupping and early head formation stage. There was a steady increase and population build-up in S1 with peaks recorded during cupping and head formation stages. High incidence was recorded

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during the pre-vegetative stage in S1, S2, S3 and S5, and during the early vegetative stage in S4 (Fig. 5.7A). There was a significant difference in spider population incidence between the different seasons ($F_{4, 20} = 128.54$; $P < .001$). Significant differences were also recorded between the plant growth stages ($F_{9,215} = 18.44$; $P < .001$), with a significant interaction between the seasons and plant growth stages in the Deciduous Forest agroecological zone ($F_{34,215} = 8.60$; $P < .001$).

Spider occurrence was first observed during the pre-vegetative stage in S1, S2 and S4, early vegetative stage in S3 and S5. The population trends showed a steady increase and decrease in spider populations across the different seasons. Peak populations were recorded during cupping and early head formation stages in S1 and S4 respectively. S3 and S3 had the same trends, with the least spider incidence throughout the crop phenology (Fig. 5.7B). There was a significant difference in spider incidence between seasons, and plant growth stages ($F_{4, 20} = 33.46$; $P < .001$ and $F_{9, 220} = 20.40$; $P < .001$, respectively), and a significant interaction between the seasons and plant growth stage ($F_{35, 220} = 7.25$; $P < .001$).

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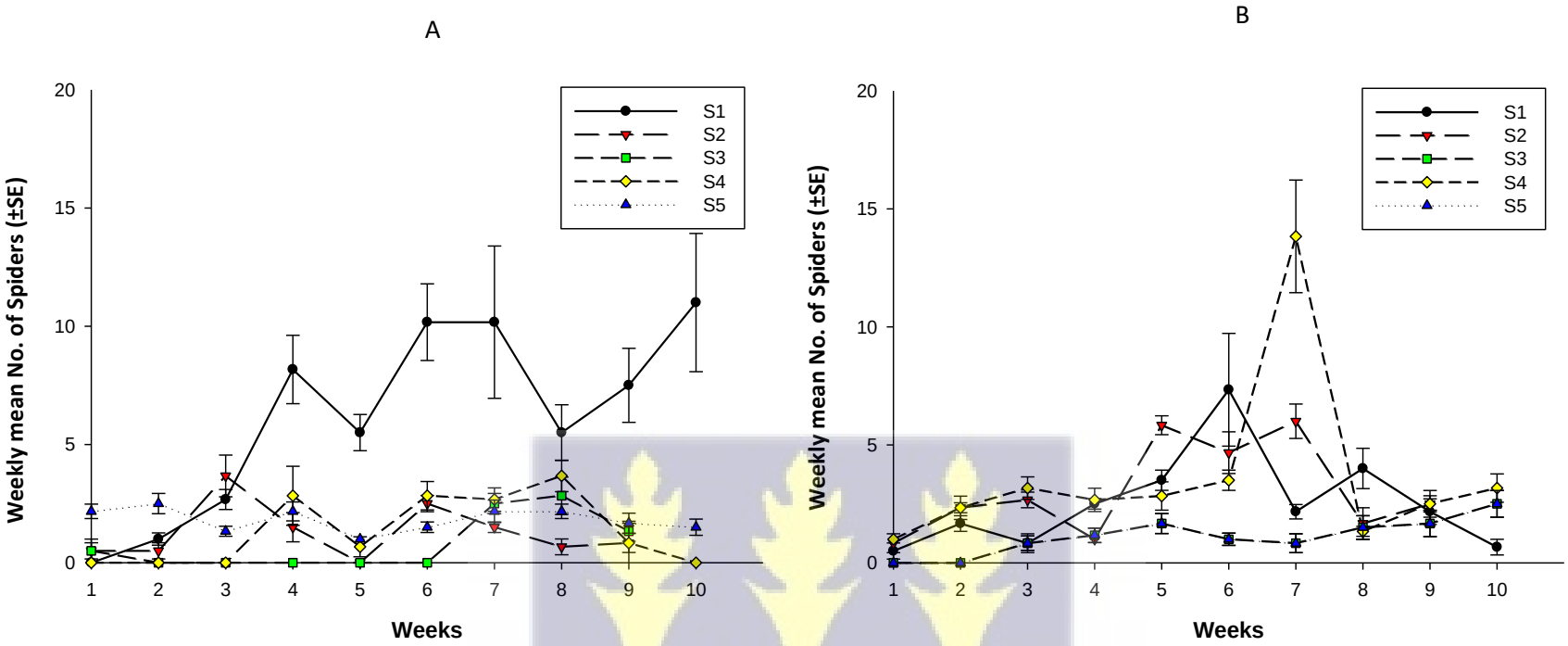


Figure 5-7: Seasonal incidence and population fluctuation of spiders (±SE) in the Deciduous Forest (A) and Coastal Savannah (B) zones across five cropping seasons (2019-2021).

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5.3.5 Factors affecting aphid abundance on cabbage

5.3.5.1 Weather parameters

In the Coastal Savannah agroecological zone, the highest density of *L. e pseudobrassicae* was observed in January (dry season) of the 2020 (year 2). Other peaks were recorded in September and October (minor rainy season) of 2020 (Fig. 5.8). In the Deciduous Forest zone, the highest densities were recorded in January (dry season) of 2021 (year 3) and other peaks were recorded in November (dry season) of 2019 (year 1).

On the other hand, *M. persicae* had very low population densities throughout the study period, with the highest densities recorded in September (minor rainy season) of 2020 in the Coastal Savannah zone, and in August (dry spell) of 2019 in the Deciduous Forest zone (Fig. 5.8). The highest population peaks of both aphids were observed to occur during periods of drought, high temperatures and lower relative humidity (Fig. 5.8).

The air temperature and RH showed a significantly negative relationship with the density of *L. e. pseudobrassicae*, and rainfall had a significantly positive relationship with *L. e. pseudobrassicae* ($P = 0.004$) in the Coastal Savannah agroecological zone (Table 5.4). No significant relationships were recorded with weather parameters in the Deciduous Forest agroecological zone

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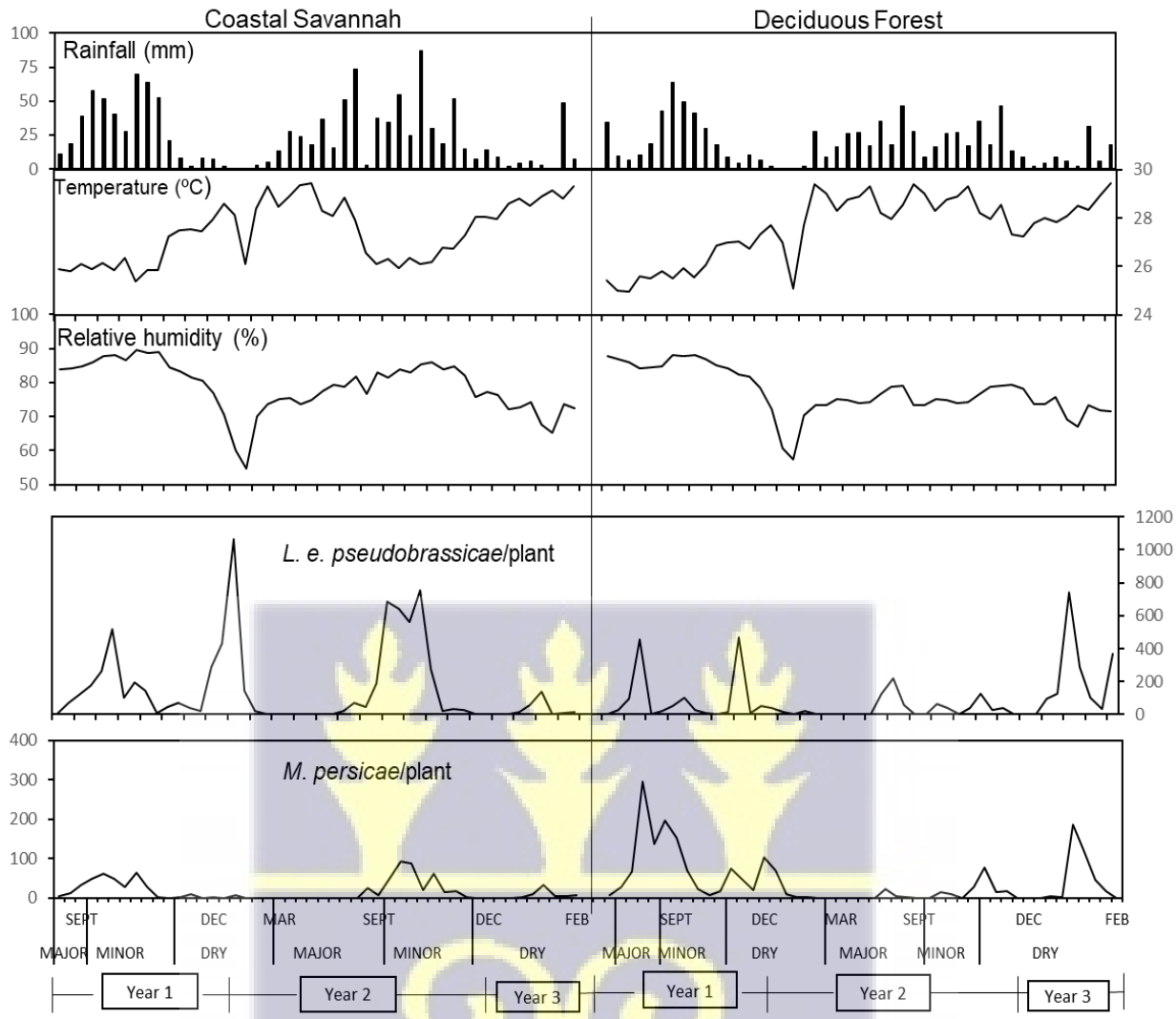


Figure 5-8 Climatic factors and aphid densities on cabbage crops in the Coastal Savannah and Deciduous Forest agroecological zones in Ghana. Values shown are the average number of individuals recorded on 20 plants

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Table 5.4: Summary of generalised linear model showing relationship between weather parameters and aphid densities on cabbage

Term	Coastal Savannah zone				Deciduous Forest zone			
	Estimate	**SE**	**Z-value**	***p***	**Estimate**	**SE**	**Z-value**	***p***
L. e. pseudobrassicae								
Intercept	26.251	5.419	4.844	**< 0.001**	36.288	32.552	1.115	0.265
Air Temperature	-0.333	0.096	-3.487	**< 0.001**	-1.132	0.768	-1.475	0.140
Rainfall	0.029	0.010	2.879	**0.004**	0.031	0.050	0.612	0.540
Relative humidity	-0.114	0.037	-3.039	**0.002**	-0.069	0.168	-0.416	0.678
M. persicae								
Intercept	8.868	7.517	1.18	0.238	20.864	13.691	1.524	0.128
Air Temperature	-0.119	0.130	-0.915	0.360	-0.364	0.330	-1.103	0.270
Rainfall	0.022	0.012	1.764	0.078	0.014	0.021	0.676	0.499
Relative humidity	-0.007	0.053	-0.133	0.894	-0.045	0.071	-0.636	0.524

Statistically significant *p*-values are boldfaced

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5.3.5.2 Natural enemies

Paragus borbonicus (syrphids) were recorded in high densities in January of 2020 (year 2) in both agroecological zones. Other peaks were recorded in September (minor rainy season) of 2019 (year 1) in both agroecological zones (Fig. 5.9). Coccinellids were generally low throughout the evaluation period, except in August (dry spell) and September (minor season) of 2019 in both agroecological zones. Peaks of Aphidoletes were recorded in September (minor season) of 2020 in the Coastal Savannah Zone and in January (dry season) of 2021 in the Deciduous Forest agroecological (Fig. 5.9). The population densities of *A. varipes* and *C. carnea* were low throughout the study period with a maximum of 3 insects per plant recorded in periods of high-density peaks in both agroecological zones. Peaks of spider density appeared irregularly throughout the study period in both zones. *Paragus borbonicus* peaks were generally observed during periods of high aphid densities in the two agroecological zones (Fig. 5.9).

Paragus borbonicus had a positively significant relationship with *L. e. pseudobrassicae* in the Coastal Savannah zone, and with both *L. e. pseudobrassicae* and *M. persicae* in the Deciduous Forest zone (Table 5.5). Coccinellids were significantly positively related while *C. carnea* was significantly negatively related to both aphids in the Coastal Savannah zone. Spiders were significantly positively related while *A. varipes* was significantly negatively related to *M. persicae* in the Deciduous Forest agroecological zone (Table 5.5).

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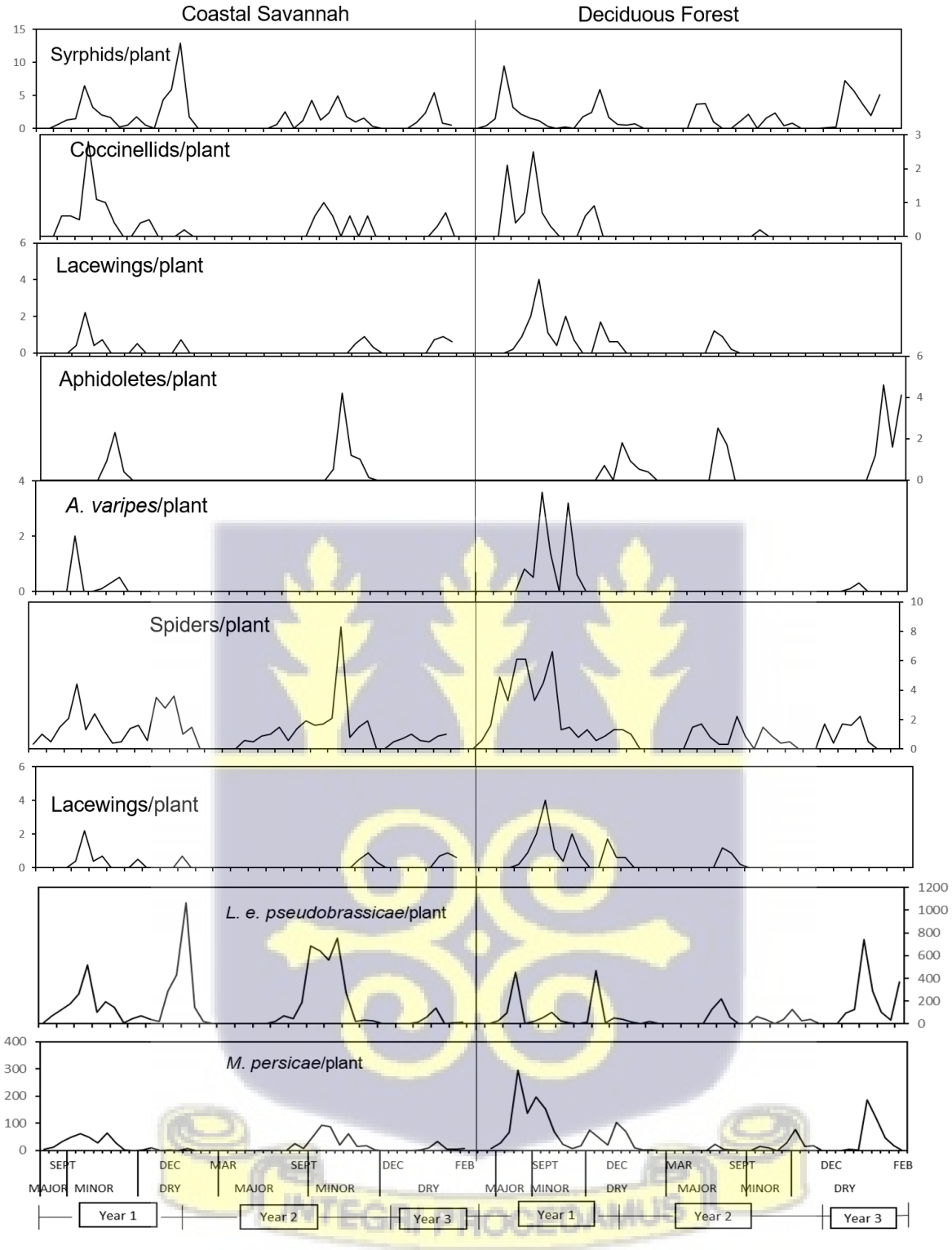


Figure 5-9 Natural enemies and aphid densities on cabbage crops in the Coastal Savannah and Deciduous Forest agroecological zones in Ghana. Values shown are the average number of individuals recorded on 20 plants

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Table 5.5: Summary of generalised linear model showing relationship between natural enemies and aphid densities on cabbage

Term	Coastal Savannah zone				Deciduous Forest zone			
	Estimate	**SE**	**Z-value**	***P***	**Estimate**	**SE**	**Z-value**	***P***
L. e. pseudobrassicae								
(Intercept)	5.426	0.338	258.092	**<0.001**	5.1	0.287	315.732	**<0.001**
P. borbonicus	0.027	0.013	4.357	**0.037**	0.042	0.010	18.628	**<0.001**
Spider	0.065	0.038	2.929	0.087	0.009	0.016	0.293	0.588
Coccinellids	0.138	0.048	8.107	**0.004**	0.044	0.052	0.696	0.404
C. carnea	-0.245	0.054	20.864	**<0.001**	0.047	0.031	2.206	0.137
A. aphidimyza	0.000	0.023	0.000	0.995	0.007	0.021	0.104	0.747
A. varipes	0.041	0.048	0.718	0.397	-0.056	0.033	2.902	0.088
***Paederus* sp**	-	-	-	-	0.014	0.057	0.059	0.807
M. persicae								
(Intercept)	3.793	0.286	176.501	**<0.001**	3.525	0.304	134.21	**<0.001**
P. borbonicus	0.021	0.012	3.228	0.072	0.043	0.011	14.784	**<0.001**
Spider	0.002	0.021	0.009	0.925	0.090	0.024	14.218	**<0.001**
Coccinellids	0.194	0.045	18.776	**<0.001**	-0.057	0.050	1.329	0.249
C. carnea	-0.154	0.051	9.308	**0.002**	0.055	0.036	2.413	0.120
A. aphidimyza	0.047	0.025	3.556	0.059	-0.033	0.024	1.806	0.179
A. varipes	0.087	0.047	3.423	0.064	-0.116	0.046	6.283	**0.012**
***Paederus* sp.**	-	-	-	-	0.070	0.049	1.996	0.158

Statistically significant *p*-values are boldfaced

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5.3.5.3 Parasitism by the aphid parasitoid; *Aphelinus varipes*

The aphid parasitoid identified during this study was *Aphelinus varipes* (Foerster, 1841) (Hymenoptera: Aphelinidae), and aphid parasitism was low in both Deciduous Forest and Coastal Savannah agroecological zones. Nevertheless, there were significant differences in the percentage parasitism recorded across the five cropping seasons ($F_{2, 12} = 33.41$; $P < 0.001$) and between the agroecological zones ($F_{1, 12} = 150.79$; $P < 0.001$). The cumulative and seasonal percentage parasitism is shown in Table 5.8. The highest seasonal percentage parasitism was recorded in S1 of the Deciduous Forest zone, and the highest cumulative percentage parasitism was also recorded in the Deciduous Forest agroecological zone (1.06%). Parasitism was generally low in both agroecological zones throughout the study (Table 5.6).

Table 5.6: Seasonal and cumulative percentage parasitism of aphids by the parasitoid *Aphelinus varipes* in Coastal Savannah and Deciduous Forest agroecological zones

Season	**% Parasitism(±SE)**	
	Coastal Savannah -Kpong	**Deciduous Forest - Aseseeso**
S1	0.48±0.07b	2.86±0.37cd
S2	0.04±0.03a	0.14±0.09b
S3	0±0a	0±0a
S4	0±0a	0±0a
S5	0±0a	1.57±0.39c
Average cumulative % parasitism	0.12±0.04a	1.06±0.23b

Different letters across rows are statistically significant at α = 0.05

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5.4 DISCUSSION

Understanding the incidence of a pest and its relationship with biotic and abiotic components (ecology) is very important, because it allows for the proper identification of critical sampling periods, environmental manipulation and implementation of pest management strategies (Ehi-Eromosele *et al*., 2013). In this study, two aphids occurred on cabbage; *Lipaphis erysimi pseudobrassicae* and *Myzus persicae* with different seasonal variation trends throughout the cabbage growth stages. *Lipaphis e. pseudobrassicae* was noted to be the more abundant aphid compared to *M. persicae*, because the former is a specialist feeder of brassicaceous crops, so tends to survive well on cabbage, while the latter is a generalist feeder, feeding on several different plant families (Radcliffe, 1982; Blande *et al*., 2004).

Different incidences were recorded for *L. e. pseudobrassicae* and *M. persicae* throughout the different cropping seasons with frequent population peaks, and in some seasons, they were found to co-exist on the field. A similar finding was reported by Fidelis *et al.* (2019), who found different seasonal variations among aphids on cabbage, with frequent population peaks across different seasons. Singh and Lal (1999) also reported inconsistencies in aphid occurrence and severity across different cropping seasons. This study also recorded a high abundance of *L. e. pseudobrassicae* on the vegetative stages of cabbage in both agroecological zones which corresponds with earlier reports by Karazmoodeh and Zandi (2013), who recorded high aphid abundance on the vegetative stage of *Brassica napus*. Notwithstanding, *L. e. pseudobrassicae* was recorded on all the different plant stages while *M persicae* was mostly found in the mature and sometimes early stages of cabbage. Elwakil and Mossler (2013), reported that *M. persicae* mostly attacks cabbage during the early head formation and heading stages of cabbage while the other

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aphids (*L. e. pseudobrassicae* and *B. brassicae)* attack cabbage at any time. This validates the importance of plant growth stages in pest management because susceptibility to pest infestation is often dependent on the plant stage, and as such, management measures must be targeted and employed accordingly (Andalaro *et al*., 1983). The population density of aphids was generally low during the third cropping season (March to early June, 2020) which falls during the major rainy season. The low population may be attributed to frequent rains experienced during this season. Hasan *et al.* (2009) reported that even the slightest rainfall was found to be detrimental to aphid population growth, and Patel and Singh (2018) reported negative effects of rainfall on aphid population buildup on cabbage.

Weather parameters play an important role in the growth, development, distribution and population dynamics of aphids (Bale *et al*., 2002; Qaisar *et al*., 2014; Skendžić *et al*., 2021). In the Coastal Savannah zone, air temperature and RH negatively affected the density of *L. e. pseudobrassicae*, while rainfall positively affected this aphid. In studies by Rao *et al.* (2013), temperature was found to be the major factor regulating aphid population on brassica plants, and temperatures recorded during the study in this zone ranged from 23.8^{o}C to 31.1^{o}C, which have been reported to be optimal for high reproduction of *L. e pseudobrassicae* (Liu and Yue, 2000). These findings also conform with Malik *et al*. (2000) and Chaudhuri *et al*. (2001), who reported a significant negative relationship between temperature and aphid populations. While rainfall positively affected *L. e pseudobrassicae* in this study, other studies have reported significantly negative relationships with rainfall on brassica (Qaisar *et al*., 2014; Mukherjee and Singh, 2017). However, although rainfall may not directly affect insect populations, it directly affects temperature and relative humidity which often has a direct effect on insect populations (Chen *et al*., 2019). None of the weather

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variables affected *M. persicae* in both agroecological zones, possibly due to this highly cosmopolitan aphid's ability to quickly acclimate to local climate conditions (Alford *et al*., 2012). Furthermore, there was minimal variation in the weather conditions in the Deciduous Forest zone, probably accounting for the non-relationship recorded between these parameters and the recorded aphid densities.

The natural enemies found to be associated with aphids have been reported in different countries (Daiber, 1971; Nieto *et al*., 2006; Gardiner *et al*., 2014; Forchibe *et al*., 2017; Kök *et al*., 2020), with syrphid flies and spiders being the most abundant in all seasons. Fening *et al*. (2020) reported a consistently high abundance of syrphid flies during a similar study on cabbage in Ghana. A similar finding was reported by Forchibe *et al*. (2017) and Fidelis *et al*. (2019) who found high abundance of syrphid flies and spiders associated with aphids on brassicaeous crops. Spiders are generalist feeders (Grez *et al*., 2014), and they were consistently recorded in this study, even in seasons with low or no aphid population. *Paederus* sp. is not a common natural enemy associated with aphids, however it was recorded to be associated with aphid infestation in the Deciduous Forest agroecological zone. Yadav *et al*. (2015) reported it as a natural enemy frequently associated with pests on cabbage. Studies by Gesraha and Ebeid (2021) reported that *Paederus alfierii* is an important natural enemy of aphids on okra in Egypt, and is also major predator of *Plutella xylostella* on cabbage (Miranda *et al*., 2011). The difference in natural enemy abundance recorded in the two agroecological zones might have been due to variation in vegetation structure and climatic conditions in these zones. Earlier studies by Aidoo *et al*. (2016), reported that variation in climate and agroecosystem vegetation structure has a huge effect on insect diversity.

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Natural enemy populations varied across the different seasons with some peaks recorded during high aphid population peaks. Of the natural enemies reported in this study, *P. borbonicus* was positively related to the densities of *L. e pseudobrassicae* in the Coastal Savannah zone, and to both aphid species in the Deciduous Forest zone. Syrphids are one of the most common predators associated with aphids (Omkar, 2016) and Honěk (1983) reported that syrphids positively respond to increasing aphid densities. Coccinellids had a significant positive relationship with both aphid species in the Coastal Savannah zone, which is consistent with Mandal and Patnaik (2006) who reported a positive relationship *L. erysimi* and *Cocinella septempunctata.* The absence of any response in the Deciduous Forest zone could be attributed to the fact that ladybugs are generalist predators (Snyder and Ives, 2003), and different species of coccinellids show diverse feeding habits (Fidelis *et al*., 2019) and may have been feeding on other pests. Fidelis *et al*. (2018) showed that syrphids and coccinellids were some of the key mortality factors of aphids on cabbage. *Chrysoperla carnea* which is known as one of the most effective generalist predators of aphids (Sarwar, 2014) presented a negative relationship with both aphid species in the Coastal Savannah zone. *C. carnea* is a generalist predator (Cheng *et al*., 2010; Sarwar, 2014) with high preference for aphids (Solangi *et al*., 2013) and has been reported to be an efficient predator of *M. persicae* (Kift *et al*., 2005). Although a positive relationship was recorded with between spiders and *M. persicae* in the Deciduous Forest agroecological zone, it should be noted that they are generalist predators, (Grez *et al*., 2014), and they were consistently recorded throughout the study period. Parasitism of aphids by *A. varipes* was low throughout the study period in both agroecological zones, however, a significant negative relationship was recorded with *M. persicae* in the Deciduous Forest zone. *Aphelinus varipes* has been reported as a potentially good biocontrol agent against *M. persicae* amongst others (Takada, 2002), nevertheless, low parasitism recorded could

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be attributed to the hyperparasitoid reported in this study *Alloxysta brevis*, which has been reported to significantly parasitise aphid parasitoids leading to low parasitoid efficiency (Sampaio *et al*., 2017). *A. varipes* is also known to kill aphids by parasitisation and by feeding on them, functioning as a parasitoid and a predator (Stary, 1988), thus, could have accounted for the low parasitism rates observed in the study. Additionally, only observed mummified aphids were counted, which could have led to an under estimation of the number of parasitized aphids, thus, could also account for the low parasitism rates reported.

The findings show that aphid and natural enemy populations fluctuate throughout different seasons, with frequent population peaks, and shows variation between the two agroecological zones. Aphid densities are affected by varying weather parameters as well as natural enemies. The important natural enemies recorded were *P. borbonicus*, coccinellids and *C. carnea* and could be explored for commercial purposes and mass control of aphids. Furthermore, the season with high rainfall (March-June; Major rainy season) recorded the lowest densities of aphids in both agroecological zones, thus recommended for cultivation of cabbage due to low pest intensity. These findings provide key insights aphid incidence and build-up on cabbage, which should be further explored for timely and efficient management of these pests.

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CHAPTER 6

6.0 Comparative bionomics of *Lipaphis erysimi pseudobrassicae* (Davis) and *Myzus persicae* (Sulzer) on three varieties of cabbage (*Brassica oleracea*) in Ghana

6.1 INTRODUCTION

The mustard aphid *L. e. pseudobrassicae* (Davis) and the green peach aphid *M. persicae* are important pests of brassica crops (Yue and Liu, 2000). Major methods of controlling this pest have focused on the use of synthetic insecticides, due to their availability and fast-acting nature despite the well-documented adverse effects of these chemicals on non-target organisms and the environment (Obeng-Ofori, 2007). Over the years, other control measures have been implemented, with regards to recent campaigns for food safety such as biological control (Yun *et al*., 2017; Ahmad *et al*., 2019), use of biopesticides (Lokanadhan *et al*., 2012; Jabran *et al*., 2016; Forchibe *et al*., 2017) and host plant resistance (Shah *et al*., 2015 Ahmed *et al*., 2018). However, the availability of resistant plant varieties is often a major setback, especially in developing countries. Screening for resistant plant lines against aphids often requires in-depth knowledge on resistant sources within crossable germplasms and associated genetics (Bhatia *et al*., 2011). However, successful trials have been conducted for resistant cabbage lines against *Brevicoryne brassicae, L. pseudobrassicae* and *M. persicae* (Shah *et al*., 2015; Ahmed *et al*., 2018). Host plant resistance is an important component of integrated pest management (IPM) and can contribute significantly to enhance crop production.

Of the different mechanisms involved in inducing plant resistance, antibiosis is the most important because it often has a direct influence on the demographic parameters of a pest such as fecundity, fertility, mortality, survival and development time (Sedaratian *et al*., 2011). Host quality often alters the demographics of pests, and this information provides important suggestions about the

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fitness of the pest on a particular host plant. It is well documented that different cultivars/varieties of a plant can alter life history attributes of herbivores by interfering with development, reproduction and consequently population growth (War *et al*., 2012; Kant *et al*., 2015; Ali *et al.*, 2021). It is, therefore, imperative to understand the life history attributes of pests on their host plants to develop ecologically sound and sustainable pest management programmes.

Life tables are powerful and essential tools for analyzing the effect of host plant quality on the growth, survival, reproduction, and intrinsic rate of increase of insect populations (Chi and Su, 2006). They facilitate the understanding of pest population dynamics by providing a complete description of the demographic parameters of an insect population under specific environments. Several lifetable studies have been carried out to investigate the effect of host plant quality on pests such as; host plant resistance of *L. erysimi* and *M. persicae* (Qayyum *et al.*, 2018; Hong *et al*., 2019), and suitability of canola varieties for *L. erysimi* (Taghizadeh, 2019). To achieve acceptable levels of food safety, it is necessary to provide and rely on more sustainable and ecologically friendly methods of pest control. Understanding the life table parameters of *L. e pseudobrassicae* and *M. persicae* on some commonly grown varieties of cabbage in Ghana will provide valuable information on less susceptible varieties for use by farmers

The focus of this study is therefore to assess the life history parameters of *L. e. pseudobrassicae* and *M. persicae* on three commonly cultivated varieties of cabbage under ambient conditions. Results obtained from this study will provide an understanding of the biological and population parameters of these two aphids on cabbage in Ghana, and serve as a baseline for understanding the role of host plants in the growth and development of both aphids.

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6.2 MATERIALS AND METHODS

6.2.1 Characteristics of cabbage varieties

Three different varieties of *B. Oleracea* var. *capitata* (Oxylus, Fortune and Leader cross) commonly grown in Ghana were used as the host plants for the study. The oxylus variety used in this study was produced by Seminis®. It is green in colour, heat tolerant, resistant to alkalinity, has soft leaves and is well adapted to a wide range of agro-ecological zones (Seminis® product catalogue). Fortune and leader cross was produced by Technisem®. Fortune has soft bluish-green leaves, resistant to fusarium wilt and is well adapted to hot and humid climates. Leader cross, on the other hand, has tough leaves, bluish-green in colour, resistant to pests and is suitable for dry seasons (Technisem® product catalogue). Different varieties of cabbage usually have different morphological features, and these varieties were used by hypothesizing that they show varied responses to aphid feeding and may also affect aphid behavior, reproduction and survival.

6.2.2 Planting of host plants

Planting of the cabbage plants was carried out at the University of Ghana Soil and Irrigation Research Center, Kpong from September-November 2020 in a screen house (Dimensions: 3.5m long, 2.5m wide and 3m high). The screen house was covered with a shade net (Organza) with a mesh size of 0.177mm (80-mesh). Seeds were obtained from Agriseed Ltd Ghana and sown in small plastic buckets (12cm x 15cm). When plants reached the third leaf stage, they were individually transplanted into 30 plastic pots (12cm x 12cm) containing a mixture of soil and Gro-Plenty organic compost (Green-Gro, Ghana) in a ratio of 2:1 as recommended by Gro-Plenty. Each variety was replicated 30 times, and the potted plants were maintained in the screen house. Irrigation was carried out daily till the end of the study. An HT-163

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barometric/humidity/temperature Datalogger (Dongguan Xintai Instrument Co., Ltd.) was placed in the screen house to monitor the temperature and relative humidity throughout the study period. Plants were used for the study when they reached the six true leaf stage.

6.2.3 Rearing of *L. e. pseudobrassicae* and *M. persicae*

Lipaphis erysimi pseudobrassicae and *M. persicae* were collected from the field in Kpong and reared on potted Chinese cabbage (*Brassica rapa*), covered with micro-perforated bread bags (Seal Packaging, Luton, UK) secured around the pots with rubber bands to restrict the aphids from moving out of the plants. The aphids were reared for three generations on each of the tested cabbage varieties to obtain a suitable population of aphids and to eliminate the possible influence of previous hosts. The aphids were transferred to new cabbage plants, by transferring individual aphids using camel hair brushes every 2 weeks, before transfer onto study plants.

6.2.4 Bionomics study

One two-day-old adult aphid was placed on a potted cabbage plant using a camel hair brush and confined in a clip cage (Plate 6.2) for each variety of cabbage. Thirty (30) aphids were used as a replicate for each variety. After 24 hours, the adult aphid and newly born ones except one were removed from the plants. This remaining aphid in the clip cage on the potted plant was kept in the screen house under ambient conditions at 30º ± 1ºC and 75 ± 5%RH and monitored daily for the various aspects of the bionomics. Nymphal instars were determined to have moulted when cast skin was observed. The developmental duration and survival of the nymphs were monitored and recorded daily until adult emergence. After adult emergence, the reproductive period, longevity, survival and fecundity of individuals were recorded daily until the death of all individuals. During

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the reproductive period, all newborn nymphs were recorded and then removed from the plant daily. The method was modified from Patel *et al*. (2017b), Qayyum *et al*. (2018) and Taghizadeh (2019). Time-specific life table parameters of aphids were generated to calculate the total survival rate, fecundity, development duration (*T*), net reproductive rate (R_0), intrinsic rate of increase (*r*), and finite rate of increase (λ).

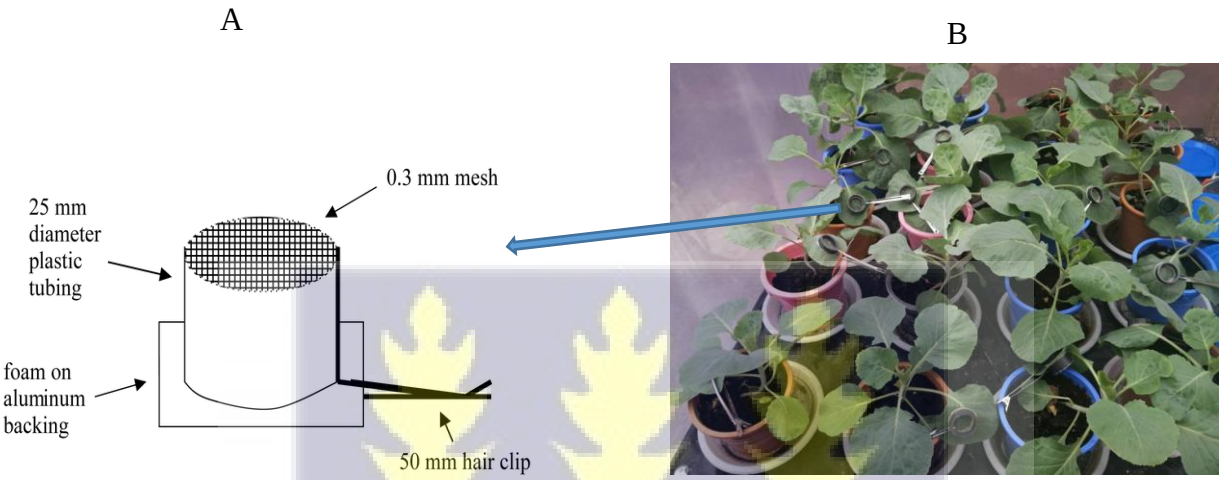


Plate 6.1: A) Clip cage used for clipping aphids B) Cabbage plants with aphids under clip cages in screen house.

6.2.5 Lifetable calculations

The developmental duration (days), survival and fecundity data were evaluated based on the female age-specific life table (Carey, 1993). Data were used to calculate the age-specific survival rate (l_x), age-specific fecundity (m_x), age-specific maternity rate ($l_x m_x$) and life table parameters (intrinsic rate of increase (*r)*; finite rate of increase (λ); net reproductive rate (R_0); and mean generation time (*T)*).

The R_0 values were calculated as:

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$$R_0 = \sum_{x=0}^{\infty} l_x m_x$$

The intrinsic rate of increase (r) was estimated using the iterative bisection method from the Euler Lotka formula with age indexed from 0 (Goodman 1982):

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1$$

The finite rate is calculated as

$$\lambda = e^r.$$

The mean generation time is defined as the length of time that a population needs to increase to R_0-fold of its population size at the stable age-stage distribution, and is calculated as:

$$T = (\ln R0)/ r.$$

6.2.6 Statistical analysis

All data for nymphal developmental duration, adult longevity, pre-reproductive, reproductive period, post-reproductive period and fecundity were subjected to Shapiro-Wilk and Bartlett tests to determine data normality and homogeneity. Data related to nymphal duration, reproductive period, longevity and total life span for both aphid species did not conform to the normality and homogeneity test, even after log transformation. Consequently, comparisons were done using the non-parametric Kruskal-Wallis test followed by post hoc comparisons of means using Bonferonni test. The fecundity life table parameters were analyzed by bootstrapping (1,000-bootstraps) with seed for Mersenne Twister set at 2.000000 to determine the standard error and the means compared

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by Tukey HSD test. Mean differences between both aphids were compared by an independent sample t-test at 95% significance level. All analysis were carried out in SPSS V22.

6.2.7 Survival analysis

Survival was analyzed and curves fitted using the Kaplan-Meier survival probabilities, followed by a pairwise comparisons Mantel-Haenszel Test (Log-Rank test) in SPSS V12. Individual aphids that did not die by the end of the nymphal period were censored (0=death event did not occur; 1=death event occurred) during nymphal survival analysis. The adult aphids were not censored, because the experiment finished with the death of all insects.

6.3 RESULTS

6.3.1 Bionomics of *Lipaphis erysimi pseudobrassicae* and *Myzus persicae*

The developmental duration for each nymphal stage, of *L. e. pseudobrassicae* and *M. persicae* on the three cabbage varieties (Oxylus, Fortune and Leadercross) are shown in table 6.1 and 6.2. Both aphids had four nymphal stages on all three cabbage varieties. Following Kruskal Wallis test, a significant variation nymphal duration was recorded for *M. persicae* (χ^2 (2) = 12.41, P = 0.001), ranging from 4.03 to 6.50 days on Fortune and Oxylus respectively (Table 6.2). An independent t-test showed a significant variation in nymphal duration between both aphid species on all three varieties (t (58) = 2.85, P = 0.006 for Oxylus, t (58) = 2.39, P = 0.021 for Fortune and t (58) = 2.19, P = 0.032 for Leadercross) with *M. persicae* recording a significantly higher duration of 6.50 and 6.40 on Oxylus and Leadercross respectively (Table 6.2).

The reproductive period and adult longevity varied significantly among varieties for both *L. e. pseudobrassicae* (χ^2 (2) = 14.82, $P < 0.001$ and χ^2 (2) = 6.97, $P < 0.001$) and *M. persicae* (χ^2 (2) =

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16.99, $P < 0.001$ and χ^2 (2) = 15.73, $P < 0.001$). The reproductive period and adult longevity were significantly higher on Oxylus variety, with a mean reproductive duration of 10.57days and adult longevity of 11.70 days for *L. e. pseudobrassicae,* and with a mean reproductive duration of 11.27days and adult longevity of 12.83days for *M. persicae* (Table 6.1 and 6.2). The adult longevity for aphids reared on Leadercross (5.23days) was low for *L. e. pseudobrassicae* (Table 6.1) and on Fortune (5.47days) for *M. persicae* (Table 6.2). A similar trend was recorded for the reproductive period of both aphid species. Significant differences in reproductive period were recorded between both aphid species on Fortune (t (58) = 2.35, $P = 0.022$) and Leadercross (t (58) = -2.49, $P = 0.016$) varieties, with *L. e pseudobrassicae* recording a higher reproductive period on Fortune and *M. persicae* on Leadercross. Longevity also varied significantly between both aphid species only on Leadercross variety (t (58) = -2.41, $P = 0.019$). Total lifespan varied significantly among varieties for both *L. e. pseudobrassicae* (χ^2 (2) = 15.52, $P < 0.001$) and *M. persicae* (χ^2 (2) = 14.59, $P < 0.001$) (Tables 6.1 and 6.2) with a significant t-test between both aphid species on Fortune (t (58) = 2.36, $P = 0.022$) and Leadercross (t (58) = -2.56, $P = 0.013$) varieties.

The fecundity of *L. e pseudobrassicae* and *M. persicae* was highly influenced by feeding on different cabbage varieties. A significant variation in the fecundity was recorded for both *L. e. pseudobrassicae* (χ^2 (2) = 23.91, $P = 0.001$) and *M. persicae* (χ^2 (2) = 24.07, $P < 0.001$). *Lipaphis erysimi pseudobrassicae* was most fertile on Oxylus variety (44.77 nymphs/female) and least fertile on Leadercross variety (12.96 nymphs/female) (Table 6.1), while *M. persicae* on Oxylus (31.27 nymphs/female) and least fertile of Fortune variety (7.67nymphs/female) (Table 6.2). Comparison between the mean fecundity of both aphid species showed a significantly higher

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fecundity of *L. e pseudobrassicae* on Oxylus (t (58) = 2.07, P = 0.043) and Fortune (t (58) = 4.93, P < .001) varieties compared to *M. persicae*.

Table 6.1: Mean of the developmental duration (days) and reproduction period of *L. e. pseudobrassicae* reared on three cabbage varieties

Parameters	Varieties (days)					
	n	Oxylus	n	Fortune	n	Leadercross
Nymphal duration						
First Instar	30	1.63 ± 0.11a	30	1.63 ± 0.11a	30	2.03 ± 0.10b
Second Instar	30	1.50 ± 0.09a	29	1.43 ± 0.11a	28	1.33 ± 0.16a
Third Instar	30	1.37 ± 0.09a	28	1.27 ± 0.13b	24	0.80 ± 0.14b
Fourth Instar	30	1.47 ± 0.09a	24	1.53 ± 0.18a	16	0.70 ± 0.13b
Adult duration						
Total nymphal duration		5.97 ± 0.13a		5.87 ± 0.37a		4.87 ± 0.43a
Adult longevity	30	11.70 ± 1.04a	24	8.60± 0.97ab	16	5.23 ± 0.97b
Reproductive period						
Pre-reproductive period		0.20 ± 0.07a		0.13 ± 0.06a		0.13 ± 0.08a
Reproductive period		10.57 ± 1.01a		7.70 ± 0.87ab		4.77 ± 0.87b
Post reproductive period		1.13 ± 0.26a		0.90 ± 0.14ab		0.47 ± 0.15b
Fecundity (nymphs/female)		44.77 ± 5.18a		31.27 ± 4.31a		12.97 ± 2.60b
Total lifespan		17.67 ± 1.02a		14.47 ± 1.13a		10.10 ± 1.32b

Means with different letter across rows significantly different by the Bonferonni test (P ≤0.05)

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Table 6.2: Mean of the developmental duration (days) and reproduction period of *M. persicae* reared on three cabbage varieties

Parameters	Varieties (days)					
	n	Oxylus	n	Fortune	n	Leadercross
Nymphal duration						
First Instar	30	1.83 ± 0.10ab	30	1.60 ± 0.16a	30	2.07 ± 0.13b
Second Instar	30	1.60 ± 0.09 a	14	0.87± 0.19b	24	1.57 ± 0.18a
Third instar	30	1.43 ± 0.09a	14	0.77 ± 0.18b	24	1.43 ± 0.164a
Fourth Instar	30	1.63 ± 0.10a	14	0.80 ± 0.18b	24	1.33 ± 0.15a
Adult duration						
Total nymphal duration		6.50 ± 0.13a		4.03 ± 0.67b		6.40 ± 0.56a
Adult longevity	28	12.83 ± 1.20a	14	5.47 ± 1.24b	22	8.73 ± 1.08b
Reproductive period						
Pre-reproductive period		0.40 ± 0.11a		0.13 ± 0.08a		0.23 ± 0.11a
Reproductive period		11.27 ± 1.30a		4.36 ± 1.11b		8.13 ± 1.03ab
Post reproductive period*		1.56 ± 0.30a		1.10 ± 0.31ab		0.60 ± 0.11b
Fecundity (nymphs/female)		31.27 ± 3.96a		7.67 ± 2.09b		14.13 ± 2.18b
Total lifespan		19.33 ± 1.24a	14	9.50 ± 1.77b		15.13 ± 1.45a

Means with different letter across rows significantly different by the Bonferonni test (P ≤0.05)

6.3.2 Lifetable parameters of *L. e. pseudobrassicae* and *M. persicae*

6.3.2.1 Survival rate

The nymphal survival rate (Mantel-Haenzel Test; $\chi 2 = 21.47$, $P < 0{\cdot}001$) and adult survival rate (Mantel-Haenzel Test; $\chi 2 = 8.19$, $P = 0{\cdot}017$) of *L. e. pseudobrassicae* differed significantly among the three varieties (Fig. 6.1, 6.2). Similar results were also recorded on the nymphal survival rate for *M. persicae* (Mantel-Haenzel Test; $\chi 2 = 23.64$, $P = 0{\cdot}001$) (Fig. 6.1). The nymphal survival rate between both aphids varied significantly on Fortune variety (Mantel-Haenzel Test; $\chi 2 = 11.35$, $P = 0{\cdot}001$) while adult survival rate between both aphids varied significantly on Fortune (Mantel-

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Haenzel Test; $\chi 2 = 5.49$, $P = 0{\cdot}019$) and Leadercross (Mantel-Haenzel Test; $\chi 2 = 7.75$, $P = 0{\cdot}005$) varieties.

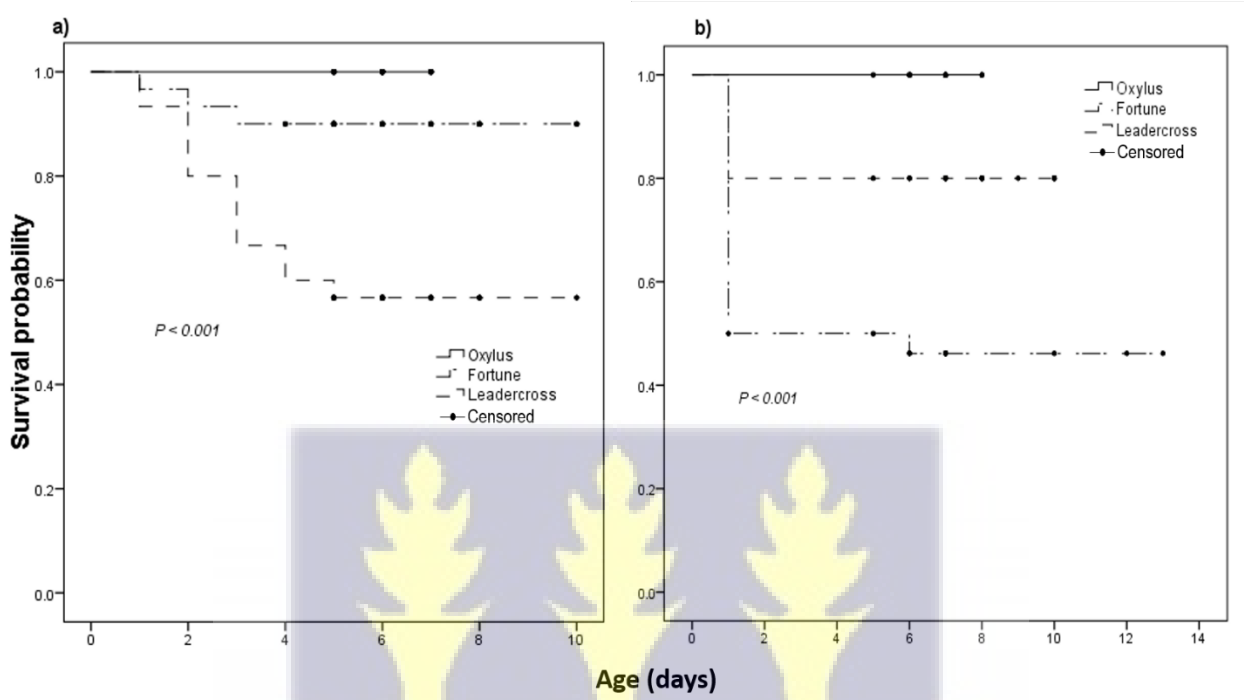


Figure 6-1: Kaplan–Meier nymphal survival curve for a) *Lipaphis erysimi pseudobrassicae* and b) *Myzus persicae* on three cabbage varieties.

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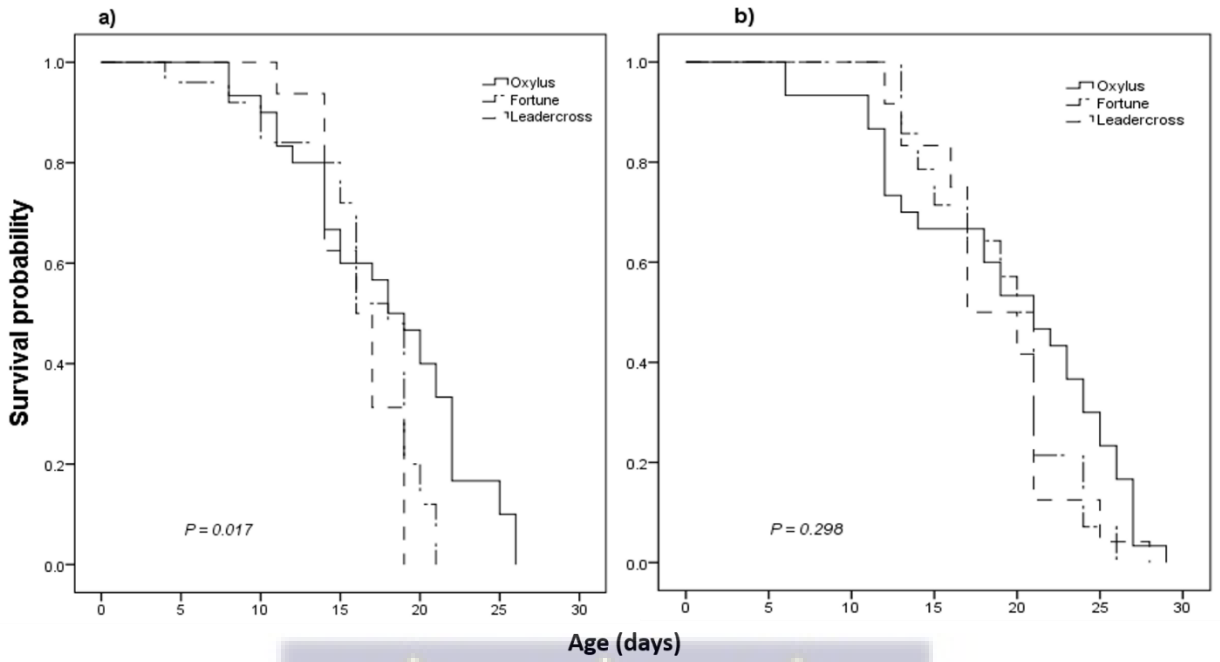


Figure 6-2: Kaplan–Meier adult survival curve for a) *Lipaphis erysimi pseudobrassicae* and b) *Myzus persicae* on three cabbage varieties.

6.3.2.2 Life expectancy

As shown in Fig. 3, the Oxylus variety revealed the highest life expectancy for both *L. e. pseudobrassicae* (e_x = 18.20) and *M. persicae* (e_x = 18.76) among the three cabbage varieties tested. In this variety, the life expectancy (e_x) was highest at the beginning of the life cycle and reached 0.0 after 26 days for *L. e. pseudobrassicae*, and 27days for *M. persicae* (Fig.6.3). Similarly, this variety revealed the highest survival rate for both aphid species. The survival rate obtained at this stage (nymphal) was 100% for both aphid species, thus recording zero mortality at the preadult stage compared to the other varieties (Fig. 6.1). The lowest life expectancy of *L.e. pseudobrassicae* was observed on Leadercross variety, which was 9.76 at the beginning of life and reached 0.0 after 19 days, while the lowest e_x for *M. persicae* was recorded on Fortune variety; 8.96 at the beginning, and reached 0.0 after 26 days (Fig. 6.3).

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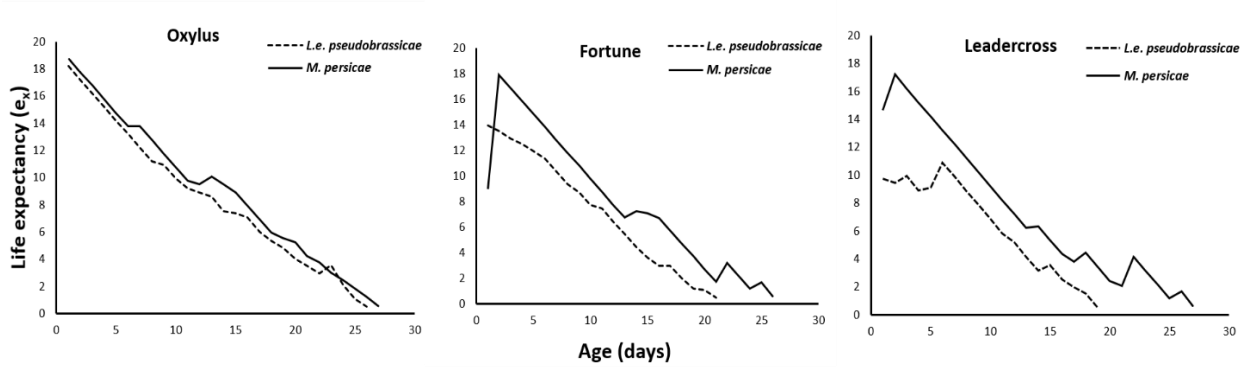


Figure 6-3: Life expectancy (e_x) of *Lipaphis erysimi pseudobrassicae* and b) *Myzus persicae* on three cabbage varieties.

6.3.2.3 Fecundity

The age-specific fecundity (m_x) peaks of both aphids on Oxylus variety were higher than those obtained from the other two varieties. Peaks were generally recorded between day 9-12 for all three varieties, for both aphid species (Fig. 6.4).

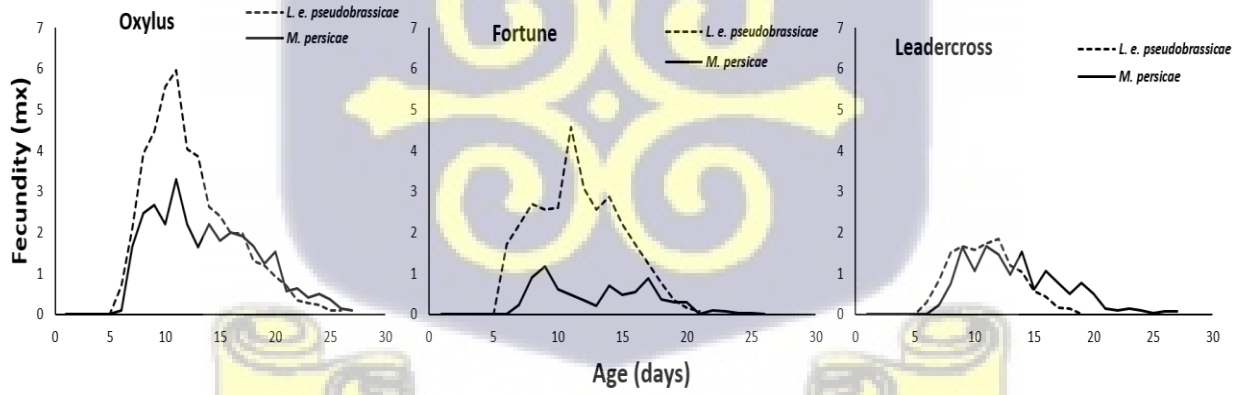


Figure 6-4: Age-specific fecundity (m_x) of *Lipaphis erysimi pseudobrassicae* and *Myzus persicae* on three cabbage varieties

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6.3.3 Population growth parameters

The population growth parameters of *L. e. pseudobrassicae* and *M. persicae* on the three cabbage varieties are as shown in table 3. Statistical analysis showed significant differences in the net reproductive rates (R_0), intrinsic rate of increase (r) and finite rate of increase (λ) among varieties for both aphid species ($P < 0.001$) (Table 3). The net reproductive rate of both *L. e. pseudobrassicae* and *M. persicae* were significantly higher on Oxylus compared to the other varieties. There was also significant difference in the doubling time (DT) ($P = 0.034$) for *L. e pseudobrassicae* among the three varieties. Between *L. e pseudobrassicae* and *M. persicae* the R_0, r and λ varied significantly on Oxylus and Fortune varieties, while R_0 (t (33) = 2.76, $P = 0.0009$) and T (t (33) = 3.13, $P = 0.004$) varied significantly on Leadercross variety.

Table 6.3 Population growth parameters of *Lipaphis erysimi pseudobrassicae* and *Myzus persicae* on three cabbage varieties

Aphid species	Parameters	Varieties		
		Oxylus	Fortune	Leadercross
L. e. pseudobrassicae	R_0 (offspring/individual)	35.96 ± 2.66a	21.89 ± 1.77b	6.46 ± 0.58c
	r_m	0.31 ± 0.02a	0.28 ± 0.02a	0.17 ± 0.03b
	λ	1.37 ± 0.02a	1.32 ± 0.02a	1.19 ± 0.02b
	T	11.47 ± 0.38a	11.15 ± 0.43a	10.70 ± 0.42a
	DT	2.22 ± 0.66a	2.51 ± 0.69a	3.97 ± 1.37a
M. persicae	R_0 (offspring/individual)	23.75 ± 1.76a	2.94 ± 0.21b	9.61 ± 0.86c
	r_m	0.25 ± 0.03a	0.09 ± 0.02b	0.18 ± 0.03a
	λ	1.28 ± 0.03a	1.09 ± 0.02b	1.20 ± 0.03c
	T	12.74 ± 0.47a	12.23 ± 0.40a	12.49 ± 0.46a
	DT	2.79 ± 0.39a	7.87 ± 6.06b	3.83 ± 1.86a

Means followed by the same letter per column do not differ by Tukey test ($P = 0.05$). *Reproductive net rate (R_0), mean generation time (T), intrinsic rate of increase (r_m), finite rate of increase (λ), Doubling time (DT)

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6.4 DISCUSSION

Life tables are useful tools to assess the effect of external factors such as host plants on the growth and development of insects (Romalho *et al*., 2015; Qayyum *et al*., 2018). In this study, it was demonstrated that the developmental and lifetable parameters of *L. e pseudobrassicae* and *M. persicae* were significantly altered by the cabbage varieties tested. Several studies have suggested that the performance of any aphid species can be greatly influenced by host plants, be it different cultivars or varieties of the same plant (Tsai and Wang, 2001). Shah (2017) reported that the quality of host plants plays an important role in the growth and development of an insect, reflecting the suitability of a particular host plant for the sustenance of an insect life cycle. The intrinsic rate of increase (r) is an important parameter used by ecologists in demographic studies of insect populations (Birch, 1948). It is often used to contrast population fitness across different climatic and food-related conditions (Tsai and Wang, 2001). Our findings also showed that the intrinsic growth rate and net reproductive rate for *L. e. pseudobrassicae* was lowest on the leader cross variety, while that for *M. persicae* was lowest on fortune variety, suggesting these hosts were not suitable for these aphids respectively, thus indicating some level of resistance to these aphids.

Oxylus was seen to be the most suitable host for both aphids, following the high values of the intrinsic rate of increase r, net reproductive rate R_0 and finite rate of increase λ. Adenka *et al* (2021) reported fortune as the most susceptible to both aphid species under field conditions, However, comparatively, the net reproductive rate, intrinsic rate of increase and finite rate of increase were higher for *L. e pseudobrassicae* compared to *M. persicae* on oxylus, which is the most susceptible variety for both aphid species. Comparatively to other aphid species, the intrinsic rate of *M. persicae* has been reported to be lower on cruciferous vegetables (Chi and Su, 2006; Hong *et al*.,

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2019), which is also comparatively lower in the current study. This can be attributed to the fact that *M. persicae* is polyphagous, and has high adaptability to complex environments and host plants (Hong *et al*., 2019), as opposed to *L. e pseudobrassicae*, a specialist aphid on cruciferous crops, thus highly adaptable for survival on the host plant used in this study.

La Rossa *et al*. (2013) suggested that nymphal duration may be directly proportional to the level of host resistance or susceptibility because high resistance levels increased the time from birth to first reproduction compared to high susceptibility. Similarly, this study recorded longer nymphal duration for the respective aphids that survived on the resistant varieties; Leadercross and Fortune. Although not investigated in the current scope of work, we speculate that high nymphal mortality recorded on these cabbage varieties might be attributable to physical or chemical properties of the host plants that negatively impacted the development of aphids. zero nymphal mortality recorded on the susceptible varieties could be attributed to favourable host plant conditions such as nutritional content. Many factors often affect the performance of aphids on different host plants, such as nutritional value, chemical composition and physicomorphic properties (La Rossa *et al.*, 2013; Jahan *et al*., 2014; Ali *et al*., 2021), and as such this might have been the case in this study.

The fecundity and longevity of aphids have also been attributed to the physical and chemical properties, as well as the nutritional value of host plants (Tsai and Wang, 2001; Ulusoy and Ölmez-Bayhan, 2006). The survival rate of both aphid species had different trends on the three cabbage varieties. The highest survival rate was recorded on the oxylus variety for both *L. e pseudobrassicae* and *M. persicae*, and the survival curves showed a preference for this variety by both aphid species, further indicating the suitability of this host to these aphids.

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Based on the performance of both aphids on the three cabbage varieties, oxylus is a more suitable host for *L. e pseudobrassicae* compared to *M. persicae*. The population buildup for *L. e pseudobrassicae* took a shorter time than *M. persicae*. Similarly, comparatively higher densities of *L. e pseudobrassicae* than *M. persicae* was recorded on cabbage throughout the five cabbage cropping seasons in the two agroecological zones, as reported in chapter five. From the findings, Leadercross variety could be used by farmers as a less susceptible variety to aphid infestation, as part of an integrated pest management approach to control aphids on cabbage. However, it would be relevant for further studies to be carried out to identify specific factors in these cabbage varieties that influence the growth of aphids.

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CHAPTER 7

7.0 Confirmation and characterization of disease-causing pathogen associated with aphid infestation on cabbage

7.1 INTRODUCTION

Cucumber mosaic virus (CMV) (a type species of genus Cucumovirus and family Bromoviridae) is an important virus because of its agricultural impact worldwide and its use as a model for understanding plant-virus interactions (Palukaitis and Garcia-Arenal, 2003; Jacquemond, 2012). It is known to be responsible for important agronomic losses in many crops worldwide, and has the broadest host range amongst known plant viruses, infecting about 1240 species of plants from different groups including food plants, ornamentals to wild plant species (Kaper and Waterworth, 1981; Edwardson and Christie, 1991; Kim *et al.*, 2011). CMV produces various kinds of symptoms on different host plants (Kaper and Waterworth, 1981). In the field it is mostly transmitted by aphids in a non-persistent manner and through seeds in some, but not all host species (Jacquemond, 2012). Palukaitis *et al*. (1992) reported efficient transmission of CMV by more than 75 aphid species, including *Myzus persicae* and *Aphis gossypii* in a stylet-borne, non-persistent manner, especially on vegetable crops. This type of transmission is usually characterized by short acquisition and inoculation times, without retention in the insect. The aphids remain viruliferous for short periods and lose the ability to transmit the virus following feeding or just a simple feeding probe. The genome of CMV is a single-stranded, positive-sense RNA having three RNA segments, which contain five genes encoding proteins designated 1a, 2a, 2b, 3a, and capsid protein (CP) (Palukaitis *et al.*, 1992; Palukaitis and Garcia-Arenal, 2003; Jacquemond, 2012). This virus has a high diversity, revealed by the large number of isolates (strains) that are both biologically and molecularly different (Jacquemond, 2012). These strains have been classified into two major

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groups by nucleotide sequence similarity, and further divisions have been made by phylogenetic analyses (Roossinck, 2003; Aramburu *et al*., 2007).

In Ghana, isolates of CMV have been reported on yams, tomatoes, cowpea (Eni *et al*., 2008). Fening *et al.* (2016), also made reports of CMV on cabbage in the Ashanti and Eastern regions of Ghana following analyses with immunodiagnostic strips. However, this isolate on cabbage has not been characterised and there is no information on its aetiology on cabbage in Ghana. This study, therefore, seeks to confirm and characterize this suspected CMV isolate on cabbage using RT-PCR, as predetermined using immunodiagnostic sticks (Fening *et al*., 2016), and to understand the disease aetiology and its relationship with aphids on cabbage. This objective was carried out under the CONNECTED Grant, in collaboration with a renowned Plant Virologist Prof. J.P. Carr of the Plant Science Department of the University of Cambridge.

7.2 MATERIALS AND METHODS

7.2.1 Collection of diseased-cabbage leaves

Sampling for diseased plants was done in the major cabbage-growing regions of Ghana (Plate 7.1). Diseased cabbage leaves (showing the characteristic symptoms of stunting, mosaic, yellowing, leaf curl and wilting) (Plate 7.1) were collected into RNA Later and stored at -20°C. Some leaf samples were dried and stored on silica gel. Samples were transported on ice to the Plant Science and Virology Department of the University of Cambridge for further studies.

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Plate 7.1: Plants showing symptoms of disease/ collection of disease plant samples

7.2.2 Bulking up virus strains in *Nicotiana benthamiana* and cabbage

Nicotiana benthamiana and *Brassica oleracea* var *capitata* plants were inoculated after 3 weeks' growth at the four-leaf stage. For virion inoculation, total RNA extracted from infected leaves, stored at -80°C was used. Two lower leaves were dusted with Carborundum (SiC) and used to abrade the leaf during mechanical inoculation to aid virus entry (Plate 7.2). A five microlitre (5ul) volume of RNA was rubbed gently onto the lower leaves and the excess of SiC was cleaned out with distilled water. The inoculated plants were grown in a walk-in growth chamber room under long photoperiod at the Department of Plant Science growth facility, University of Cambridge botanic garden. The light intensity was 200µE.m^{-2}.s^{-1} (using Sylvania Activa 172 professional 36W bulbs) and temperatures maintained at 20-22°C. Watering was done automatically on a 36h watering regime for plants. The plants were left to grow for at least 10 days and were inspected for phenotypic symptoms of the disease. Leaves of both cabbage and benthamiana were tested for CMV using the CMV Agristrip in Agristrip buffer. Further evidence of the presence of the virus was by RT-PCR.

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Plate 7.2: Inoculation of (i) *N. benthamiana* and (ii) cabbage plants at the Plant Science Department, University of Cambridge

7.2.3 CMV Agristrip test

Leaf discs were cut out from inoculated *N. benthamiana* and *B. oleracea* and placed in 1.5ml centrifuge tubes, containing 200µl (enough to grind leaves) of the agristrip extraction buffer. A micro pestle was used to grind the leaves, then spinned for 30 seconds. CMV test strips were then placed into the liquid and observed after 2 minutes for the positive red line

7.2.4 Pathogen Identification

7.2.4.1 RNA extraction

To extract RNA, approximately 50mg of leaf samples stored in RNA later were used. The leaf was patted on tissue paper to remove any excess RNA Later. The leaf was placed in a sterile microcentrifuge tube containing 600 µl of RL buffer. 25 µL of 2-mercaptoethanol was added to the microcentrifuge tube and a micropestle was used to grind until the sample was homogenized. The homogenate was centrifuged and the lysate was transferred into an RNAse-free microcentrifuge tube using a pipette. The lysate was spun for two minutes to pellet any debris. The

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resulting supernatant was transferred into another RNAse-free microcentrifuge tube and stored on ice.

7.2.4.2 Genomic DNA removal

gDNA removal columns were assembled and 600µL of the lysate was applied onto the column and centrifuged at 14,000xg (-14000RPM) for one minute. We ensured that all the lysate goes through into the collection tube by inspecting the column. If the entire lysate did not pass through, spinning was done again for an additional minute. The flowthrough was maintained and stored at -20°C, until RNA purification. The gDNA removal columns with bound gDNA were disposed of.

7.2.4.3 Total RNA purification

To every 100µL of flowthrough, 60µL of 96-100% Ethanol was added and the mixture was vortex. RNA purification columns were assembled, and 600µL of the lysate with ethanol was applied onto the column and centrifuged at ≥3500 x g (-6000 RPM). It was ensured that all the lysate went through into the collection tube. The column was washed by applying 400µL of wash solution A according to the manufacturer's instruction and centrifuged for 1 minute. The flowthrough was discarded and the spin column was reassembled with its collection tube. The last two steps were repeated and the column was washed for the third time. The flowthrough was discarded and the column spun again for 2 minutes to thoroughly dry the resin. The collection tube was discarded. The column was now placed in fresh 1.7 mL elution tubes provided with the kit. 50µL of elution solution A was added to the column and centrifuged for 2 minutes at 200 x g, followed by 1 minute at 14000 x g. the resulting purified RNA is stored at -80°C till cDNA synthesis.

7.2.4.4 Analysis for RNA concentration and purity

For all experiments, RNA was analysed for concentration and purity using Nanodrop® ND1000 spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Sterile nuclease-

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free water was used a blank and 1µL of the RNA from each sample was used in quantification. The ratio of absorbance at 260nm to absorbance at 280nm was determined, with an absorbance of 1.8-2.0 considered indicative of high purity standards. RNA was stored at -20°C pending further downstream manipulation.

7.2.4.5 Reverse transcription Reactions

RNA was reverse transcribed using GoScript™ (Promega). Viral RNA (upto 500ng) in 1µL was mixed with 1µL (0.5µg/µL) of Random Primer (Sigma) and topped up to 5µL with nuclease-free water (random primers will amplify different portions of the genome). The mixture was incubated at 70°C for 5 minutes and placed on Ice to allow the primer to anneal to the viral RNA. The Goscript™ reverse transcription mix was prepared according to the manufacturer's instructions (4µl GoScript™ 5X reaction Buffer, 3µl $MgCl_2$ (final concentration 2.4mM), 1µl dNTP mix, 1µl ribonuclease inhibitor (final concentration 1.33U/µl), 1µl GoScript™ reverse transcriptase enzyme (final concentration 0.333U/µl were mixed and topped up to 15µl with nuclease-free water) and mixed with 5µl of RNA and primer mix made in the initial step. The mix is annealed at 25°C for 5 minutes, extended at 42°C for 60 minutes and then the reverse transcription enzyme was inactivated by incubating at 70°C for 15 minutes. Tubes containing cDNA were placed on ice for use in the PCR using specific primers.

7.2.4.6 PCR Reactions

Primers were purchased from Sigma-Aldrich for known sequences as indicated in Table 7.1, and each primer was diluted according to the manufacturer's instructions and used in separate PCR reactions to screen for viral pathogens present in plant samples. Following positive gel plates for cucumber mosaic virus (CMV) coat protein, complete genome sequencing was done using primers indicated in appendix 5.

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PCR was carried out using 2X Biomix Red® (Bioline, London, Uk) premix [contains: BIOTAQ DNA polymerase (5U/µl), 2mM dNTPs, 32mM (NH4)2SO4, 125mM Tris-HCl (pH 8.8 at 25°C), 0.02%Tween 20, 3mM MgCl2 stabiliser, inert dye] (Bioline) according to the manufacturer's instructions. 10µl of the premix was mixed with 9µl of nuclease-free water, 1µl of the cDNA template (50ng/ µl) 0.5 µl of forward and reverse primers. PCR cycling conditions were initial denaturation 95°C for 3 minutes followed by 30 cycles at 94°C for 30seconds, an annealing step at 54°C (or appropriate temperature), and extension step at 72°C for 1 minute and a final extension of 72°C for 10 minutes on a thermal cycler (ABI-Applied Biosystems, Veriti, USA).

7.2.4.7 Gel electrophoresis of DNA

The DNA amplicons generated by PCR were loaded into wells of a 1% (w/v) agarose gel in TAE (Tris-Acid EDTA) buffer [0.04M Tris, 1mM EDTA pH 8.0, 0.1142% (v/v) glacial acetic acid] containing 0. 05µg.ml^{-1} ethidium bromide. The gels were submerged in TAE buffer and run in an MHU-1010 gel rig (Flowgen/Scientific Laboratory supplies, Yorkshire, UK) at 100V using a power-pac 3000 (Bio-Rad, Hemel Hempstead, UK). Ethidium bromide-stained gels were examined to reveal bands under UV illumination on an Alphalmager® gel documentation system (Alphannotech/ProteinSimple, Santa Clara, CA, USA) depending on the expected amplicon size, an appropriate size marker (either 1kb ladder or 10kb ladder) was loaded adjacent to the samples.

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Table 7.1: Primers used to test for the presence of virus in cabbage leaf samples

	Group/Virus	Primers	Sequence (5'-3')	Author
1	CMV Coat protein, RNA 1, RNA 2.	CMVCPF	GTGTGTCGAGTCGTGTTGTC	JP Lab
		CMVCPR	ACCCTTCAGCATCAGTGGAA	
		RNA 1F	CCACCCGAACTCATTCGACATT	
		RNA 1R	TTTCCTCGCGGSGTCTCAAAG	
		RNA 2F	GAAGCTAAGGTGATGGAACCTTGC	
		RNA 2R	AGCGGTRTACTTCTTAAASGCGC	
2	Tobamoviruses	TobamodF	TKGAYGGNGTBCCNGGNTGYGG	Li *et al.*, 2019
		Tobamod R	ACNGAVTBNABCTGTAATTGCTAT	
3	Generic potyvirus	Nib2F	GTITGYGTIGAYGAYTTYAAYAA	Zheng *et al.*, 2008b
		Nib3R	TCIACIACIGTIGAIGGYTGNCC	
4	Generic Polerovirus	PococpR	CGTCTACCTATTSGGRTTN	Zhou *et al.*, 2011
		PoconF	TGYTCYGGTTTTGACTGG	
5	CMV CP to distinguish subgroup I and II	CMVIIF	CTACGTTTATCTTCC	Chen *et al.*, 2011
		CMVIIR	AACCGGTGATTTACCATCGC	
		CMVIF	GCCACCAAAAATAGACCG	
		CMVIR	ATCTGCTGGCGTGGATTTCT	
6	*Brassica Yellows virus*	BrYVR	TCA GGA AGA AAC ATT CGA GTC C	Lim *et al.*, 2015
		BrYVF	CTT ACA TTG AAA TTC TAA AGA G	

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7.2.4.8 Sequencing

Before sequencing, gel positive PCR products were diluted to the required concentration specified by the sequencing provider (Source Biosciences Lifesciences, Nottingham, UK). All the amplicons were sequenced in both the forward and reverse directions using the respective forward and reverse primers used to generate them by using sequencing techniques developed by Sanger *et al*. (1977) and automated by Smith *et al*. (1986).

7.2.5 Phylogenetic analysis and pairwise comparison

Relevant sequences for phylogenetic analyses were downloaded from GenBank in FASTA format (Appendix 6). Multiple sequence alignments were done using MUSCLE (Edgar, 2004) in Molecular Evolutionary Genetics Analysis version 6.0. (MEGA 6) (Tamura *et al.*, 2013). The evolutionary history was inferred using either the neighbor-joining method under the Kimura-2 parameter with 1000 bootstraps (Saitou and Nei, 1987). MEGA 6 was used to determine the most appropriate substitution model for the dataset. Pairwise comparisons for similarity were done by aligning the sequences using MUSCLE in Species Demarcation Tool v 1.2 (SDT v. 1.2) (Muhire *et al.*, 2014).

7.2.6 Aphid/disease interaction on cabbage

7.2.6.1 Sampling for disease incidence, severity and associated aphids

Sampling for disease incidence was done alongside sampling for aphids. Sampling sites were the same as reported in Section 3.1 of Chapter 3. Three farms per district were observed for disease incidence. Field observations were carried out to monitor aphid/disease incidence, and disease

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severity in 2019 and 2020 at Kpong (Lat. 6.134874, long. 0.08207). Land preparation, nursery establishment, and transplanting were done as described in Sections 4.2.2 to 4.2.4 of Chapter 4, and the field was laid out in 6 plots. To assess the disease incidence and severity, all plants were monitored weekly, after two weeks of transplanting for the entire growth period of the crop (10 weeks). Plants showing disease symptoms (stunting in young plants, leaf curl, mosaic, necrosis) were tagged and the aphid population was monitored and recorded till the death of plants. Time of disease incidence and disease severity in relation to aphid incidence were also recorded

7.2.7 Data analysis

Simple linear regression was carried out to determine the relationship between aphid abundance and percentage incidence/severity. All analyses were carried out in GENSTAT V. 12.0.

7.3 RESULTS

7.3.1 Identification of cucumber mosaic virus (CMV) on cabbage

RT-PCR showed a positive test for cucumber mosaic virus (CMV) coat protein for diseased-leaf samples collected from Kpong and Kumasi. Positive gel electrophoresis revealed bands for samples at 400bp, consistent with the number of base pairs for the CMV coat protein as indicated in Plate 7.3.

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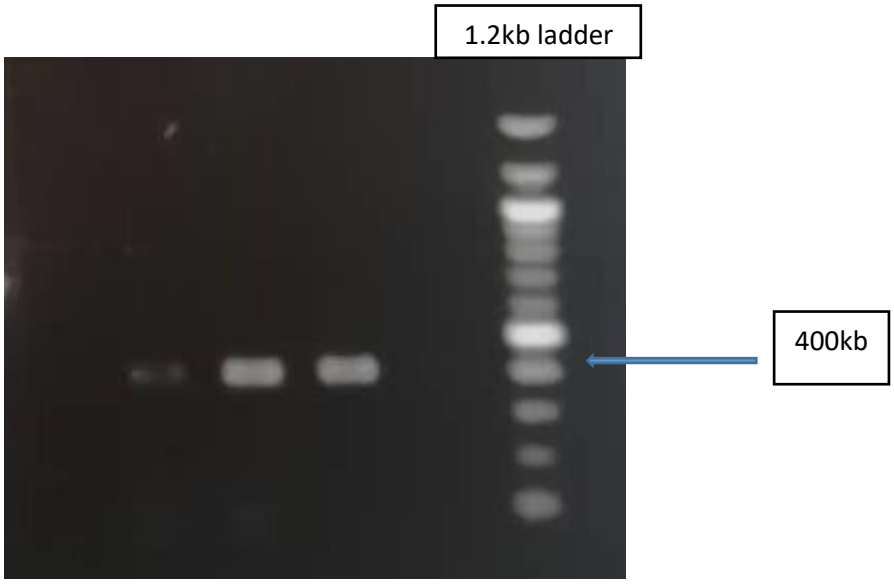


Plate 7.3: Gel electrophoresis showing 400bp fragment of the CMV coat protein gene

7.3.2 Characterization and phylogenetic analysis of the cucumber mosaic virus genome

The genome of the identified cucumber mosaic virus was sequenced and is comprised of three RNAs. The portion of the sequenced RNA 1 comprised of ≈ 1842 base pairs, RNA 2 comprised ≈ 280bp, and RNA 3 comprised ≈ 2069 bp. BLAST homology using the NCBI BLAST tool showed that RNA 1 of the CMV-Gh strain showed 99.2% similarity to the Fny strain, from the USA, RNA 2 showed 99.13% similarity to an S-strain from South Africa, and RNA 3 showed 95.89% similarity with the PV0473 strain from Italy, which is a naturally occurring pseudo-recombinant.

In the phylogenetic analysis, the RNA 1 sequence of the CMV-GH (highlighted in the dash box) showed the closest similarity to RNA1 of subgroup IA strains and clustered with the Fny-strain (Fig. 7.1). Pairwise comparisons revealed the same results with a similarity to the Fny strain of

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subgroup IA from the USA. The RNA2 sequence of the CMV-GH (highlighted in the dash box) clustered in the same clade with subgroup II CMV strains. Pairwise comparisons CMV-GH RNA 2 showed the closest similarity to the S-strain (group II) from South Africa (Fig. 7.2). RNA 3 sequences clustered in the same clade with subgroup IB CMV strains (Fig. 7.3). Pairwise comparisons of the RNA 3 of the CMV-GH strain showed the closest similarity to the PV0473 strain of subgroup I B from Italy. Phylogenetic analysis for RNA 1, RNA 2 and RNA 3 showed the same results from NCBI BLAST.

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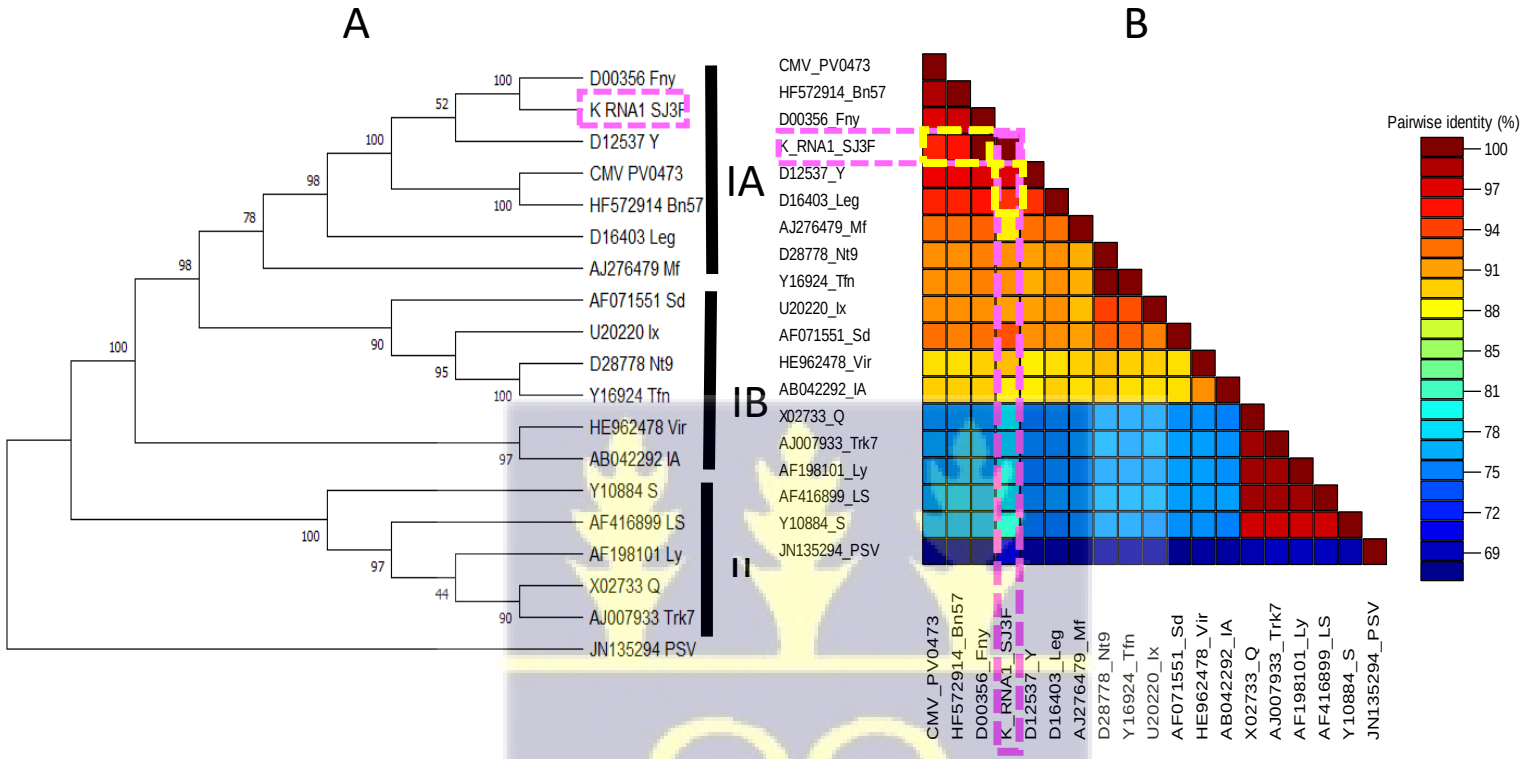


Figure 7-1: Phylogenetic analysis of CMV using selected RNA1 sequences from GenBank and CMV Isolate GH (K RNA1 SJ3R).

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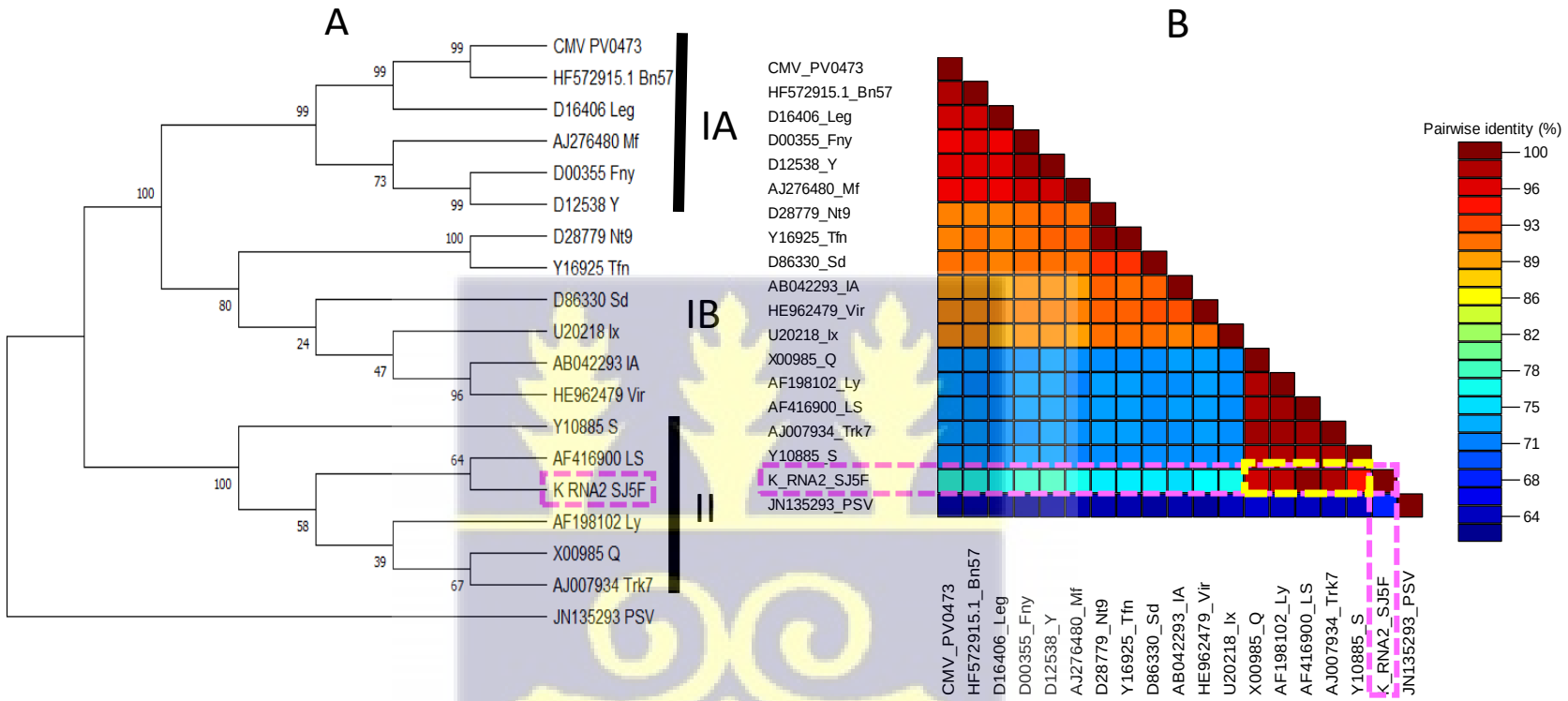


Figure 7-2: Phylogenetic analysis of CMV using selected RNA2 sequences from GenBank and CMV Isolate GH (K RNA2 SJ5F).

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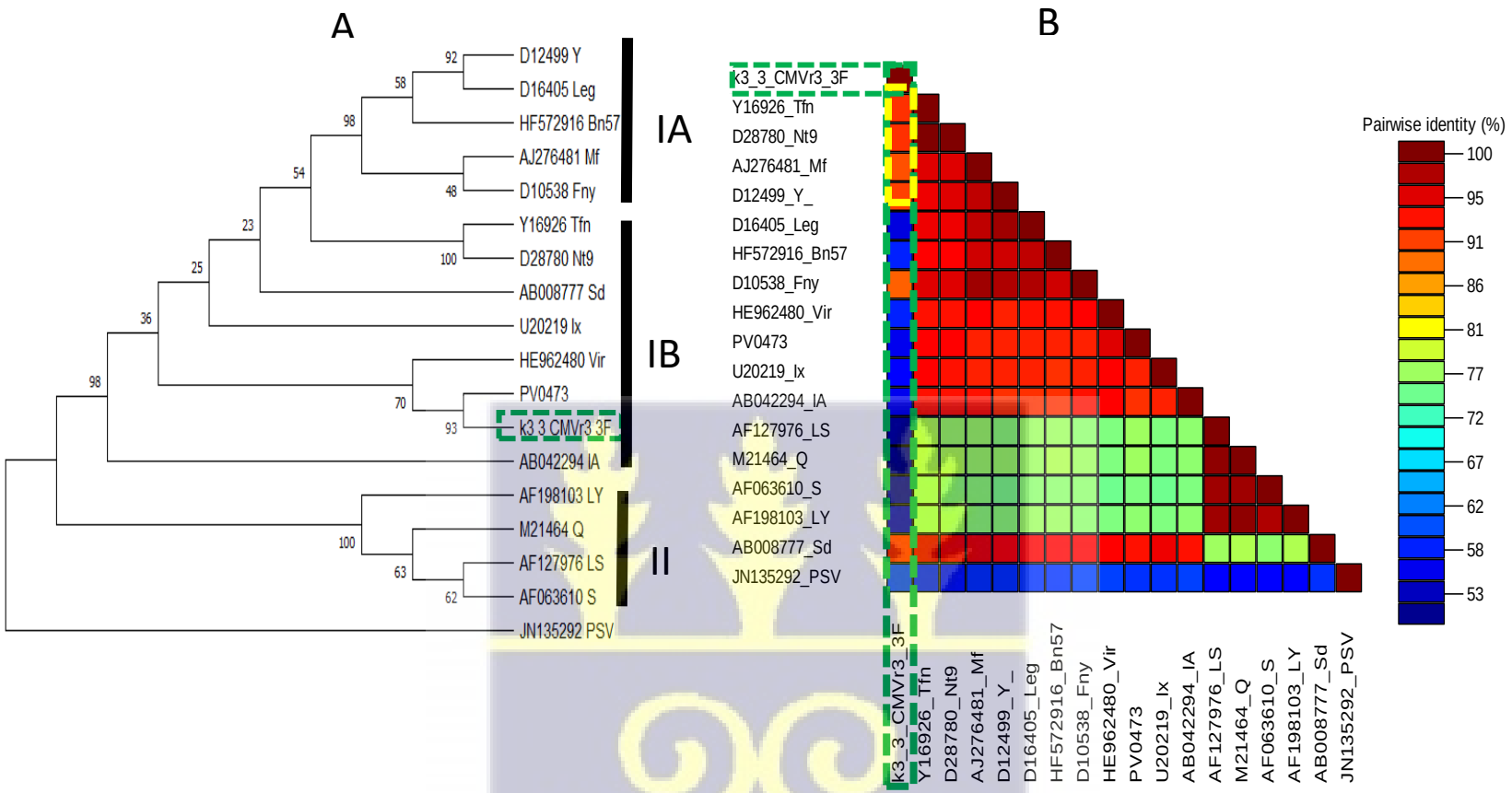


Figure 7-3: Phylogenetic analysis of CMV using selected RNA3 sequences from GenBank and CMV Isolate GH (K3 3CMVr3 3F)

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7.3.3 Transmission experiments

No sign and symptoms of the disease on field cabbage was observed on inoculated *N. benthamiana* and *B. oleracea* up to five weeks after inoculation. Analysis with CMV immunodiagnostic Agristrips (Plate 7.4) and RT-PCR showed negative for CMV coat protein.

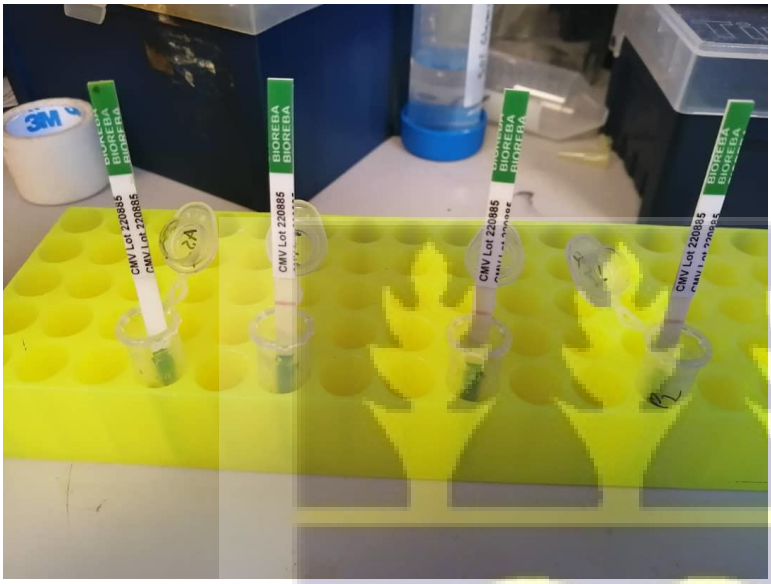


Plate 7.4: Negative immunodiagnostic strips for CMV antibodies

7.3.4 Relationship between aphid incidence and disease onset

The CMV-GH strain is characterized by stunting, mosaic, mottling and necrosis of plant tissue. Disease incidence was observed in the minor rainy seasons, throughout the sampling period. From observations and monitoring, disease presence was only recorded after aphid presence was recorded 100%. Typically, the onset of disease symptoms was recorded two weeks after observation of aphids. The disease was found to be associated with a higher abundance of *L. e.*

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pseudobrassicae, compared with *M. persicae*. Plants with disease symptoms were found to be infested with *L. e. pseudobrassicae.*

7.3.5 Relationship between aphid abundance, viral disease incidence and severity

Simple linear regression between disease severity and abundance of aphids on diseased plants revealed that there exists a significant relationship between disease severity and abundance of *L. e. pseudobrassicae* ($F_{(1, 18)} = 57.96$, $P < .001$) and *M. persicae* ($F_{(1, 18)} = 5.09$, $P = 0.037$). There was a strong significant negative correlation (r = -0.87) between disease severity and *L. e. pseudobrassicae* abundance per observed infected plant. A similar significant relationship was recorded with *M. persicae*, but the correlation was fair (r = -0.47) compared to *L. e. pseudobrassicae* (Appendix 7). However, the regression analysis further showed that there was no significant relationship between percentage disease incidence with *L. e pseudobrassicae* ($F_{(1, 16)} = 1.62$, $P = 0.221$) and *M. persicae* ($F_{(1, 16)} = 0.72$, $P = 0.407$) abundance.

7.4 DISCUSSION

This study confirmed *cucumber mosaic virus* (CMV) as the pathogen associated with the observed necrotic disease on cabbage (CMV-Gh). CMV is the type species of the genus Cucumovirus in the family Bromoviridae, and was first described in 1916 by Dolittle in Michigan as a disease of cucurbits (Palukaitis and García-Arenal, 2003). The virus is capable of infecting a large number of plants and has been isolated from over 500 naturally occurring species (García-Arena and Palukaitis, 2009). Moreno *et al*. (2004) reported CMV in other brassica crops such as broccoli, co-occurring with the beet western yellows virus. In the same study, cabbage lines screened were found to be infected with beet western yellows virus and cauliflower mosaic virus. Erkan *et al*.

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(2013) reported CMV on cabbage for the first time in Turkey, Liu *et al*. (2017a) reports naturally infecting CMV on celery cabbage in Shandong province of China for the first time. In a study by Sevik and Deligoz (2017), fifty cabbage lines were screened for infection with cucumber mosaic virus, eight cabbage lines were found to be moderately susceptible and three were highly susceptible to CMV infection. This further confirms that CMV has a broad host range including crops of the brassica family, particularly cabbage. It is also worth noting that infected cabbage leaves from Kpong and Kumasi were tested for the presence of Cauliflower Mosaic Virus (CaMV), Turnip Mosaic Virus (TuMV), Beet Western Yellows Virus (BWYV), and Cucumber Mosaic Virus (CMV) using RT-PCR in 2016, and CMV was the only virus present (Fening *et al.*, 2016).

The genome of CMV comprises three RNAs; RNA 1 (≈3,379bp), RNA 2 (≈3,038bp) and RNA 3 (≈2,162bp) (García-Arena and Palukaitis, 2009). In this study, only portions of the RNAs of the CMV-Gh genome were successfully sequenced, with RNA 1 (1842bp), RNA 2 (280bp) and RNA 3(2069bp). From the number of base pairs sequenced, concrete conclusions can only be drawn when more sequences are available. However, sequencing and phylogenetic analysis show that this CMV-Gh has originated by the exchange of genomic RNA components between three distinct CMV strains (pseudo-recombinant). RNA 1 is most similar in sequence to RNA 1 molecules of Subgroup IA CMV strains, RNA 2 is derived from a Subgroup II strain, and RNA 3 from a Subgroup IB strain. Subgroup IB CMV strains are most common in Asia (Moury, 2004). Thus, CMV-Gh is a hybrid virus likely to have arisen as the result of re-assortment of genomic RNAs of indigenous strain(s) and an unwittingly imported CMV strain(s) giving rise to a novel virus able to efficiently infect cabbage. Recombination of CMV viral strains often leads to enhancement of symptom severity (Phan *et al*., 2014). This could have accounted for the severity of symptoms

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observed on cabbage plants. The Subgroup IB RNA 3 encodes a viral coat protein that is less well detected by commercially-available immunodiagnostics (Chen *et al*., 2011).

Passaging experiments following Koch's postulate to confirm CMV-Gh as the causal pathogen proved unsuccessful, suggesting that it is still unclear if CMV is the causal pathogen of the observed symptoms on cabbage. However, there is strong evidence that suggests CMV is pathogen associated with the manifestation of the necrotic disease. Nevertheless, other schools of thought suggest that the identified virus could be associated with other viruses coupled with environmental factors and aphid infestation to produce the observed effect on cabbage. The observed disease symptoms which are characterized by mosaic, mottling, chlorosis, wilting and subsequent death of plants regardless of their age, with no doubt look like a viral infection, however cannot be solely attributed to CMV, since understanding CMV-Gh's role in the manifestation of the disease seems to be difficult due to the negative results of the passaging experiments which failed to demonstrate Koch's postulates. Additionally, Aphids are also known to cause stunting, leaf curl and sometimes yellowing to death of plants during extreme infestations, and diseased plants had a high infestation of *L. e. pseudobrassicae*. Chlorosis on cabbage can also be caused by *Fusarium oxysporum* sp. conglutinans, which leads to premature leaf drop, and plants remain stunted (Okungbowa and Shittu, 2012; Liu *et al*., 2017b); a similar characteristic observed in the expression of the disease on cabbage.

The observed disease was found to be associated with infestation of *L. e. pseudobrassicae* and *M. persicae*. The results showed that the disease was only present after aphids had been recorded, suggesting a relationship between aphids' presence and the onset of disease. Cucumber mosaic

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virus is transmitted by *M. persicae* on several host plants in a non-persistent manner (Palukaitis *et al*., 1992; Gildow *et al*., 2008; Tungadi *et al.*, 2017). *Lipaphis erysimi* is also reported as a non-efficient transmitter of CMV (Tian *et al.*, 2012). Further analysis showed a negative correlation between the abundance of both aphids and disease severity, suggesting that an increase in disease severity may be associated with a decrease in the number of aphids. This is consistent with findings that CMV-infected plants tend to ward off aphids to efficiently spread the pathogen to newer plants (Safari *et al*., 2019; Wamonje *et al*., 2020). Tungadi *et al*. (2021) reported that CMV induced a feeding deterrence against *M. persicae*, and also reduced their growth and development when confined to infected plants. The study further iterated that, this form of resistance caused by CMV inhibits prolonged phloem-feeding but promotes the acquisition of virions by aphids, as it encourages probing of plant epidermal cells. The growth and development of *L. erysimi* confined on the same CMV-infected plant were not affected (Tungadi *et al*., 2021). This can also explain why infected plants had a high infestation of *L. e. pseudobrassicae* compared to *M. persicae.* The current study reports the occurrence of cucumber mosaic virus on cabbage for the first time and the phylogenetic relationships of CMV subgroup IB isolate in Ghana.

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CHAPTER 8

8.0 Farmers' perception of aphids as pests of cabbage and their associated damage

8.1 INTRODUCTION

Cabbage is a very popular vegetable grown in Ghana mainly by smallholder vegetable farmers in urban and peri-urban areas, serving as a source of livelihood for both rural and urban dwellers (Owusu-Boateng and Amuzu, 2013; Abdulai *et al.*, 2017). Because of its high demand and several health benefits, cabbage cultivation is a very lucrative business, especially when grown during the offseason from December to April (Ofori *et al*., 2021). The growth of cabbage thrives in moist climatic conditions, and in Ghana, it is mostly grown in the South (Greenroot, 2021). Several varieties of cabbage are grown in Ghana and farmers' choices are often dependent on the length of the crop cycle and head size, to meet early market demands (Adeniji *et al*., 2010).

Cabbage cultivation is faced with a myriad of pests, which often leads to a reduction in the market value and sometimes total crop loss in severe infestation (Mochiah *et al*., 2011). Among the complex of pests that attack cabbage, aphids are of major importance because their attack often leads to severe crop losses (Forchibe *et al*., 2017; Adenka *et al*., 2021). In addition, they are noted for their role in viral disease transmission (Ng and Perry, 2004). Aphids often pose major issues to farmers during crop production and significantly reduce the yield of crops. In objective one of this study (Chapter 3), two aphids were identified; *L.e pseudobrassicae* and *M. persicae*, with the former being the most abundant across all the surveyed regions. Due to massive damage caused by these pests on cabbage, previous research has shown that farmers rely on conventional pesticides to solve pest-related issues (Baidoo *et al*., 2012). Liu and Sparks (2001) reported that aphids were one of the most damaging pests encountered by farmers during the cultivation of

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cabbage. Farmers' perception of pest bioecology and status is important to mitigate damage caused by pests on cabbage (Oladejo *et al*., 2021). Understanding indigenous farmers' knowledge, their perception on pest challenges and their resilience strategies related to pest problems on cabbage are relevant in designing tailored pest management approaches that meet farmers' needs. Thus, this objective sought to assess farmers' perception of aphids as pests of cabbage, their associated damage, and the control measures employed by farmers to manage them.

8.2 MATERIALS AND METHODS

8.2.1 Administration of questionnaires

To assess the impact of aphid damage on cabbage, questionnaires were administered to 121 farmers in Southern Ghana which is confirmed as the major cabbage growing sector in Ghana (Greenroot, 2021). Questionnaires were administered in the Central, Ashanti, Eastern and Greater Accra regions of Ghana. The questionnaire sought to understand farmers' perception of aphids as pests of cabbage, the impact of this pest on cabbage production, and their measures of control. The consent of all respondents was sought before questionnaires were administered.

8.2.1.1 Interviews

Interviews were conducted from November 2020 through April 2021 using a semi-structured questionnaire and appropriate responses were ticked/filled following the responses of farmers (guided questionnaires). The interviews commenced with demographic parameters and socio-economic status of correspondents which included; age, sex, level of formal education, sector of professional training in agriculture and number of years of experience in cabbage production. The

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knowledge and perception of aphids were assessed on the identification, first appearance on the field, seasonal occurrence, levels of infestation, damage associated with the infestation, methods of control and cost of control in each cropping season.

8.2.1.2 Knowledge and perception of aphids and their damage to cabbage

Respondents were asked questions on the identify, occurrence and infestation of aphids on cabbage. Information about their seasonal trend, symptoms of their presence and actual damage was assessed. Respondents were asked to score the damage caused by aphids when no control measure was applied and when control measures were applied. Finally, respondents were asked to identify other pests which co-occur with aphids on cabbage, stating their damage and the intensity of the damage they cause. A pictorial key of pests associated with cabbage and their damage was used alongside the questionnaires to aid respondents in the identification of aphids and to confirm their associated damage.

8.2.1.3 Management practices

Farmers' knowledge of control measures employed in cabbage production against pests was assessed. Respondents were asked the control methods employed during the course of cabbage production and the efficiency. Due to the high rates of pesticide use in vegetable cultivation reported in Ghana (Afari-Sefa *et al.*,2015; Aniah *et al*., 2020), respondents were assessed on their knowledge and perception of pesticides used in cabbage production. Questions explored the types of pesticides used from 2019 to 2020, frequency of application during a cropping season, the reason for application, efficiency and frequency of change of the pesticides used.

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8.2.2 Data analysis

Descriptive statistics were carried out to group farmers' responses using SPSS V.22.

8.3 RESULTS

8.3.1 Demographics of respondents

Questionnaires were administered to a total of 121 cabbage farmers from November 2020 to April 2021. Three age classes of respondents were recorded; 3.3% were between the ages 26-35, 95.9% between the ages 36 to 45, and 0.8% were older than 45 years. About 90.9% of the farmers were males, while 9.1% were females. About 57.0% of farmers had formal education, while 43% had no formal education, with 85.1% having completed basic school and about 14.9% have completed secondary school. A good number of farmers (52.9%) had received formal training in agriculture related fields; 99.2% had training in crop production and protection, 18.2% in pest management, 66.9% in food safety and 8.3% in marketing. The farming experience of farmers ranged from 1-2 years to greater than five years. About 16.5% of farmers had 1-2years of experience, 54.5% had 3-5 years of experience, and 28.9% had more than five years of experience. Most farmers had more than one farm, ranging from 1 to 2 acres in size. 16.5% had 1 farm, 51.2% had two farms and 32.3% had more than 3farms. 48.8% of these farms were 1 acre in size, 43.0% were about 2acres and 7.4% were 3 acres and above.

Table 8.1: Demographic parameters of respondents.

Parameter	Frequency	Percentage (%)

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Sex		
Male	110	90.9
Female	11	9.1
Age		
20-35 years	4	3.3
36-45years	116	95.9
>45 years	1	8
Education		
Basic	103	85.1
Secondary	18	14.9
Tertiary	0	0
Agricultural training		
Yes	64	52.9
No	57	47.1
Type of training		
Crop production	63	99.2
Pest management	22	18.2
Food safety	42	66.9
Marketing	10	8.3
Experience in cabbage production		
1-2 years	20	16.5
3-5 years	66	54.5
>5 years	35	28.9
Number of farms		
1 farm	20	16.5
2 farms	62	51.2
>3 farms	39	32.3
Size of farms		
1 acre	59	44.8
2 acres	52	43
3 acres	9	7.4

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8.3.2 Knowledge on cabbage production

Cabbage was grown in different seasons across the different regions in which the questionnaires were administered. In the Central region, 17.1% of farmers grew cabbage in the major rainy season, 8.6% in the minor rainy season, 65.7% in the dry season and 5.7% all year round. In the Ashanti region, 45.9% cultivated cabbage in the major rainy season, 24.3% in the minor rainy season and 20.7% in the dry season. In the Eastern region, 31% grew cabbage in the major rainy season, 13% in the minor rainy season, 20.7% in the dry season and 34.5% all year round. In the Greater Accra region, 35% cultivated cabbage in the major rainy season, 10% in the minor rainy season, 35% in the dry season and 20% all year round. About 91.4%, 94.6%, 69% and 75% of farmers responded that the dry season was the best season for cultivation of cabbage in the Central, Ashanti, Eastern and Greater Accra regions respectively. The major cabbage varieties grown included: oxylus, fortune, kk cross, super cross, santa, Copenhagen market and leader cross. The percentage of the cultivation of the different varieties is shown in Fig. 8.1. All farmers indicated that their reason for venturing into cabbage production was because it was very lucrative.

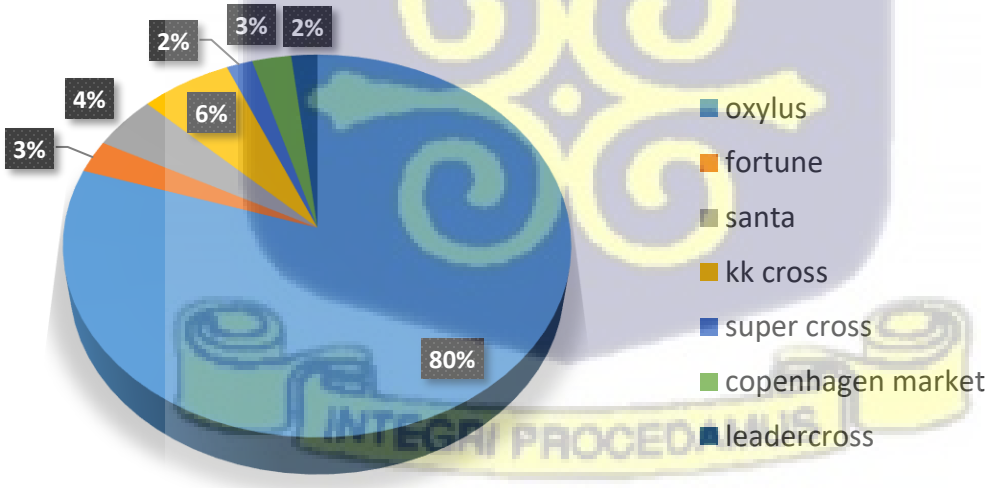


Figure 8-1: Percentage cultivation of different cabbage varieties.

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8.3.3 Knowledge of aphids and their damage on cabbage

All farmers interviewed knew about aphids and could identify them on the field and also on the pictorial key provided. Two aphids were identified; the green (*Lipaphis erysimi pseudobrassicae*) and pink aphids (*Myzus persicae*), locally called 'nkokodwe' (plant lice) by farmers across all the regions sampled. About 97.5% of farmers observed that every year, aphids first occur on the field in June, while 98.5% of farmers said July. A majority reported both June and July. All farmers knew that aphids were associated with damage on cabbage. Nearly forty percent of farmers reported that aphid infestation on the field usually starts 2 weeks after transplanting, 52.9% reported 3 weeks after transplanting and 7.4% reported from the nursery. Listed symptoms associated with aphid damage ranged from stunting, yellowing, wilting, unmarketable heads, and only 7.6% of farmers reported that these symptoms were also associated with other pests. Approximately 91% of farmers reported that these damage were solely attributed to aphid infestation. Other pests reported by farmers to be associated with damage were snails (17.6%), *Pieris rapae* (16%), *Plutella xylostella* (48%) and *Hellula undalis* (18.4%). Pictorial keys were used to aid farmers in identifying other pests. The highest aphid population density was reported to be observed in the dry season in the Central (94.3%), Eastern (58.6%) and Greater Accra (60%) regions, and in the major season (83.8%) in the Ashanti region. Cumulatively, 56.2% of farmers reported that the highest aphid population density was observed during the dry season. Farmers reported estimated damage from 5% to about 50%. Estimated damages of between 0-5% was reported by 5% of farmers, 10-20% damages were reported by 72.7% of farmers, 30-50% damage were reported by 14.0% of farmers, and 8.3% reported estimated damage above 50%.

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8.3.4 Management practices

Farmers employ different management practices to control insect pests. All farmers interviewed reported that they spray pesticides once they see aphids on the field. They further iterated that, if they don't spray, they will have up to 100%losses. All the farmers used synthetic insecticides, however, 11.6% used synthetics together with biological insecticides. Farmers used different types of pesticides as shown in Table 8.1. The major classes of insecticides used were organophosphates, neonicotinoids, pyrethroids and biological insecticides. The responses also revealed that insecticides such as Ema star®, K. optimal®, Golan®, Bipel®, and Best farmer® were the most commonly used insecticides for aphid control on cabbage. About 93% of farmers applied insecticides in the morning, while 6.6% applied in the evening. 59.5% of farmers wore protective clothing before the application of insecticides while 40.5% did not. Furthermore, in Ashanti region, 86.5% of the farmers did not wear protective clothing before applying pesticides.

Though farmers apply a wide range of pesticides, about 70.2% of the farmers reported that these insecticides were not effective in controlling the aphids, thus resorted to personal augmentation of insecticides such as increasing the dosage or application rate (41.1%), increased frequency of spraying (12.5%), mix different groups of insecticides (cocktail) (44.4%) and alternate insecticides used (2%). Although farmers usually receive free pest management advice from extension agents, some farmers prefer to consult private experts (Wuepper *et al*., 2021). However, in this study, no farmer reported seeking expert advice in cases of non-effectiveness of pesticides, the reason being that consulting an expert is expensive. About 31% of farmers reported that it would cost them between 200-500 Ghana cedis to consult an expert, while 44.3% reported that it would cost them between 500-1000 Ghana cedis. Nevertheless, they further reported that they spend approximately

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the same amount on pest control, through the purchase of pesticides; about 58% spend approximately 200-500 Ghana cedis on pest control per cropping season.

Table 8.2: List of pesticides used against aphids on cabbage in Southern Ghana.

Common name (Trade name)	Active ingredient	Frequency	Percentage (%)
Agoo®	55% Bt + Monosultap	6	5.0
Bipel®	*Pieris rapae* granulosis virus + *Bacillus thuringiensis*	68	56.2
Emastar®	Emamectin benzoate + Acetamiprid	112	92.5
Best Farmer®	Imidacloprid	27	22.3
Golan®	Acetamiprid 200g	79	65.3
Lambda®	Lambda cyhalothrin	4	3.3
Adepa®	Ethyl palmitate	6	5.0
K. optimal	Lambda cyaholothrine + Acetamiprid	39	32.2
Dursban®	Chlorpyrifos 480g	8	6.6
Anti-Atta® (Akape®)	Imidacloprid	12	9.9
Protect®	Emamectin Benzoate 19.2g	10	8.3
Officer toro®	Acetamiprid	15	12.4
Nova-Bt®	*Bacillus thuringiensis*	6	5.0
Polythrin C®	Cypermethrine and Profenetos	3	2.5

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8.4 DISCUSSION

Cabbage is a very popular leafy vegetable mostly grown in the urban and peri-urban areas by men (Timbilla and Nyarko, 2004). The study showed that only about 10% of farmers were females, while the remaining 90% were males, with a dominant age group between 20 to 45 years. This domination by males in the production of cabbage might be attributed to the fact that cabbage production is labor-intensive, with frequent watering, weeding and application of insecticides to control pests. Amoako *et al.* (2014) also reported male dominance in cabbage production in the forest zone of Ghana, attributing it to the same reasons. Male dominance of about 98% in leafy vegetable production in Ghana was reported by Quansah *et al*. (2020). The observed male dominance could also be attributed to the societal definition of gender roles in agriculture, that farming is a man's job since it involves 'manpower' to carry out most tasks such as land preparation and irrigation which are often done manually for small scale cultivation (Hope *et al*., 2009).

More than 50% of farmers had no formal education, which was also reported by Badii *et al.* (2013) and Amoabeng *et al.* (2017) that, most cabbage farmers in Ghana have a low level of formal education. Farmers that had some formal education also undertook other training courses in agriculture and had a better understanding of pesticide use. This is confluent with studies by Amoabeng *et al*. (2017) who found that farmers who had some training in agriculture had good agricultural practices compared to those who did not have any training (Amoabeng *et al*., 2017). Nevertheless, years of experience in cabbage production also played a major role in skill acquisition among farmers.

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Cabbage farmers usually do not own land, and the land tenure systems practice is leasehold, hiring and share-cropping amongst others, which accounts for the cultivation on several farms with small sizes (Osei *et al*., 2013). This study also recorded cultivation on multiple farms with average farm sizes less than an acre, which could be because of difficulties in acquiring or owning land. Timbilla and Nyarko (2004) also noted that land acquisition for the cultivation of cabbage was a major problem for farmers.

The choice of cabbage variety for cultivation was oxylus. Several studies reported oxylus as the most popular variety of cabbage grown in Ghana (Osei *et al*., 2013; Amoabeng *et al*., 2017). This variety is known as the 'the variety of choice' for most farmers, who often express reluctance in growing other varieties because oxylus produces compact and heavier heads. However, Timbilla and Nyarko (2004) reported kk cross as the most popular vegetable grown by farmers, owing to the fact that it is early maturing and produces bigger heads. He further iterated that most farmers preferred oxylus because it produced heavier heads with longer shelf life compared to kk cross.

Although about 80% of farmers reported the dry season was the best time for cabbage cultivation, most of them cultivated in the rainy seasons especially in the Greater Accra, Eastern and Ashanti regions. Farmers reported that growing cabbage in the dry season is more lucrative, because of scarcity and high demand, while growing in the rainy season is less lucrative, albeit accompanied by low pest density. In the Central, Greater Accra and Eastern regions, a good number of farmers grow cabbage all year round to meet the ever-growing demand in the urban cities. These farmers reported that cabbage has a short growing period, and a quick turnover can be achieved within a short time. Other farmers even noted that 'cabbage is the most lucrative vegetable to grow'.

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Aphids are a well-known pest of cabbage in Ghana and were reported by most farmers to cause between 30-50% damage, and more if untreated. High aphid infestations often lead to stunting and sometimes complete death of crops and unattended fields could have up to 100% loss (Mc Cullum *et al*., 1992; Mersha *et al*., 2014). Farmers reported that the first occurrence of aphids on the field was between June to July, and infestation generally starts about two to three weeks after transplanting. This same scenario was reported in field studies undertaken and reported in chapter 5, where for most cropping seasons, aphid infestation was recorded 2 weeks after transplanting. Forchibe *et al*. (2017) also reported aphid infestation on cabbage two weeks after transplanting. Damage symptoms reported by farmers included stunting, yellowing, wilting, sooty mold, which were consistent with known symptoms associated with aphid infestation (Mckinley, 1992). Farmers further reported that high aphid population density is usually observed during the dry season, due to the dry weather. Aphid growth and development are greatly influenced by environmental factors, thus in suitable conditions, populations thrive. The dry season is usually accompanied by high temperatures and a decrease in humidity, suitable for the rapid reproduction of aphids (Hughes, 1963). Narjary *et al.* (2013) reported that the mustard aphid population increased as the relative humidity decreased. Other pests identified by farmers were *P. rapae, P. xylostella, H. undalis* and snails all of which have been reported as key pests of cabbage in Ghana (Mochiah *et al*., 2011; Ngosong *et al*., 2021).

Pest management in Ghana among vegetable growers has been highly dependent on pesticides (Ntow *et al*., 2006; Amoako *et al* 2012; Quansah *et al*., 2020). In this study all farmers relied on pesticides for aphid control, stating that it is fast and can kill all insects within a short time. All farmers used synthetic pesticides, with only a small proportion alternating with biological

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pesticides. The most common pesticides used by farmers has been consistent over the past years as reported by Amoah *et al*. (2006) Amoako *et al*. (2014) and Amoabeng *et al.* (2017), so it is not surprising that a large percentage of farmers reported that the pesticides were not effective and resorted to other means to augment pesticide action. To deal with issues of resistance, some farmers increased the dosage of pesticides, stating that "the stronger the poison, the more potent it is" while others mixed different pesticides, and the rest increased spray frequency or alternate different pesticides. These findings correspond with Mattah *et al*. (2015) who reported that farmers mixed different types of pesticides to control pests because they believed the mixture had a high potency. Amoako *et al*. (2014) also reported a high percentage of farmers increasing pesticide dosage when recommended dosages were ineffective. The negligence recorded among farmers to use protective covering before pesticide application has long been reported in other studies. Several farmers complained that wearing protective clothing is not comfortable, which is also reported in a study by Amoabeng *et al*. (2017) to be one of the major reasons that farmers do not wear protective clothing.

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CHAPTER 9

9.0 GENERAL DISCUSSION

9.1 INTRODUCTION

In most parts of sub-Saharan Africa, rural households derive their income mainly from agriculture, and the rapid growth of rural populations is putting pressure on governments to create jobs (Aberman and Lal, 2019). In Ghana, agriculture employs more than 40% of the population and contributes about 19.7% to the country's GDP (Ministry of Economy and Industry, 2020). About 90% of farm holdings are less than two hectares, owned by smallholder farmers who account for the greatest share of agricultural production (Adu *et al*., 2018). In 2016, vegetable cultivation made up only a small part of the crops sector (GhanaVeg, 2014), however, with the recent emergence of a large middle class, the demand for local and exotic vegetables has increased. Consequently, the national vegetable market alone is growing by more than 10% per year (Ministry of Economy and Industry, 2020). Of the numerous varieties of exotic vegetables produced in Ghana, cabbage (*Brassica oleracea* var capitata) is widely grown, and its cultivation serves as a source of livelihood to urban and peri-urban smallholder farmers (Cofie *et al*., 2003; Otchere *et al*., 2020). Cabbage has a high demand, with a huge urban market due to the wealth of health benefits associated with its consumption (FAO, 2020). However, the devastating impacts of pests and diseases have led to difficulty in production and signify a serious threat to smallholder farmers' livelihood. This is typified with pests such as aphids, which not only cause direct crop losses but also vector viral diseases to plants, and account for one of the major constraints faced by farmers. Oftentimes, calendar spray regimes are applied with no knowledge of pest species and bioecology (Nansen and Ridsdill-Smith, 2013), notwithstanding, it is fundamental to identify aphid species on cabbage to adequately understand host-plant and vector-viral relationships to

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underpin vector and disease management interventions. Furthermore, pest biology and bioecology also play an important role in the development of pest management interventions.

This thesis addresses aphid bioecology on cabbage, to understand aphid-cabbage and aphid-virus relationships under varying seasonal and climatic conditions. Adding to the literature extensively discussed in chapter 2 of this thesis on aphids as pests of cabbage, their diversity, bioecology and economic impacts were reviewed thereof. The review clearly showed that three major aphids occur on cabbage; *Lipaphis erysimi pseudobrassicae, Brevicorryne brassicae* and *Myzus persicae*, causing significant crop losses, and with varying distribution trends across the world (Chapter 2). This study further provides supporting evidence to the varying distributions of aphids associated with cabbage in Ghana, with relevant information on their bioecology, which underpins baseline information for decision making. This section of general discussion focuses on the synthesis of information to provide further insight into aphid diversity and occurrence on cabbage and their relationship with some biotic and abiotic factors in Ghana. It further discusses the implications of these findings on aphid control on cabbage, in the broader perspective of attaining food security and for further research.

9.2 Diversity and distribution of aphids on cabbage in Ghana

This chapter of the thesis determined the species of aphids present on cabbage and their distribution across regional and agroecological zones. In addition, the regional abundance and spatiotemporal variation of the aphids on cabbage were further evaluated. We mapped the distribution of the identified species in the Upper East, Northern, Bono East, Brong Ahafo, Ashanti, Greater Accra, Eastern, Central and Volta regions; the nine regions sampled in Ghana. The findings revealed two

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major aphids occur on cabbage, *Lipaphis erysimi pseudobrassicae* and *Myzus persicae*, which corroborates findings from other studies that reported *L. e. pseudobrassicae* and *M. persicae* as key aphid pests on cabbage (Liu and Sparks, 2001; Forchibe *et al*., 2017; Fidelis *et al*., 2019; Gouvêa *et al*., 2019). Fening *et al.* (2016) reported that cabbage farmers in Ghana usually incur huge yield losses due to aphids either directly or by the death of plants caused by a viral pathogen. Although, some regions were not sampled, for example, Western and Western North regions, which are reported to produce a lot of cabbage (Crouch, 2001; MoFA, 2018), their climatic conditions characterized by average temperatures of 25º to 31ºC (Ghana Meteorological Agency) are suitable for *L. e. pseudobrassicae*. We speculate the presence of this aphid and the highly polyphagous *M. persicae* in these regions. Nevertheless, contrary to the findings of this study, other studies on cabbage across Ghana reported the cabbage aphid *B. brassicae* as the major aphid on cabbage (Fening *et al*., 2011; Baidoo *et al*., 2012; Osei *et al*., 2013; Ngosong *et al*., 2020). It is likely, they may have confused the false cabbage aphid (*L. e. pseudobrassicae*) with the cabbage aphid (*B. brassicae*), as both species closely resemble each other (Fening *et al*., 2016).

Furthermore, investigations on the abundance and spatiotemporal variation revealed that although both aphid species were recorded in all the agro-ecological zones in this study, there was significant variation in the abundance of these insects across these zones. This confirms reports that agroecological barriers have a significant effect on insect distribution and abundance (Faheem *et al*., 2019). According to IFPRI (2009), agroecological zones are geographical areas that have similar climatic conditions and vegetative cover. This accounts for the zonal variation observed in this study, as it was reported in Fajinmi *et al*. (2011) that aphid abundance is influenced by agroecological vegetative cover and climatic conditions. These findings show that aphids would

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have different pest intensities across different climatic zones, which can be attributed to their reliance on environmental conditions for their growth and development. As all regions in Ghana could not be sampled during this study, this led to the studies in chapter four, where we used ecological modelling to map suitable habitats for *L. e. pseudobrassicae* and *M. persicae.* The findings can be further confirmed with actual occurrence data in the recommended suitable habitats.

9.3 Aphid spatial pattern and habitat suitability

Species distribution models (SDMs) have been used in several studies across the world to map pest habitats, and these generated results are credible for use in informed decisions (DUeroski, 2009; Fiaboe *et al*., 2012; Sultana *et al*., 2017; Cuéllar *et al*., 2020). SDMs are statistical models of relationships between species and their environment, based on occurrence data and measures of environmental factors, that limits species distribution (Guisan and Thuiller, 2005; Elith and Leathwick, 2009; Elith and Franklin, 2017). In chapter 4, species distribution modelling was used to model the habitat suitability of *L. e. pseudobrassicae* and *M. persicae*, and also identify the important environmental variables that limit their distribution in Ghana. The model revealed different habitat suitability sites, which were confirmed by the available occurrence data of both aphid species. It further revealed that temperature and isothermality were the most important weather variables that affected the distribution of *L. e. pseudobrassicae* and *M. persicae.* Several species distribution modelling of insect pest has shown similar results obtained in this study, with temperature being one of the most relevant variables (Fiaboe *et al*., 2012; Sultana *et al*., 2017; Yeh *et al*., 2021). The use of SDMs and GIS tools in entomology has gained huge popularity over the last decade and has been shown to play a key role in pest management and resource allocation

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(Cox and Vreysen, 2005; Beckler *et al*., 2005; Fiaboe *et al*., 2012; Solhjouy-Fard *et al*., 2013; Sciarretta and Trematerra, 2014; Tonnang *et al*., 2015; Taucare-Ríos *et al*., 2016; Albuquerque *et al*., 2018; Saha *et al*., 2021). Per the findings in this study and others, it is clear that these tools are important in pest forecasting, mapping pest risk invasion routes, especially with the current trends of climate change. Combining SDM with explicit data on pest population dynamics (example, in chapter 5 of this thesis), may improve aphid forecasting which can be useful for vegetable farmers in Ghana.

Bebber *et al*. (2019) reported that several unidentified crop pests and pathogens are presumably already present. As invasive pest species threaten global food security, pest risk analysis is relevant in quarantine and biosecurity efforts (Venette and Hutchison, 2021). This study provides a predictive model for the current distribution of *L. e pseudobrassicae* and *M. persicae* based on climatic factors, which can be adapted for future prediction patterns at the national level. With the frequent band of vegetable export to the European Union (EU) from Ghana, due to potential harmful invasive pest species (Bragard *et al*., 2019), SDM can be a useful tool in informing the climate suitability patterns of associated pest species, which is relevant in determining pest risk invasion. Bebber *et al*. (2019) provided in-depth studies on such a model for global pest distribution. Additionally, data on host presence can also increase the efficiency of a model, for example, Dutta *et al*. (2008) precisely determined pest affected areas, when satellite-based remote sensing data were used to map the regional spatial distribution of *L. erysimi* in India. He further concluded that the availability of satellite remote sensing data helps in precise and timely prediction of areas with high pest intensity. Früh *et al*. (2018) reported that remotely sensed-based predictions significantly improved the prediction of pest species.

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These findings show that SDM can inform researchers about particular ecological adaptations of aphids and other insect pests in targeted areas, which can optimize efficiency and cost in pest control programmes. Dicko *et al*. (2014) reported that using SDM to map tsetse fly distribution reduced the cost of control and increased efficiency. Nevertheless, uncertainties in predicting species distribution under climate change have been discussed (Meynard *et al*., 2013). These tools offer the opportunity of using real-time host data, pest occurrence and climatic data to understand pest behaviour patterns in relation to their environment. The findings of this study provide additional evidence that SDMs can be used in understanding pest bioecology, and to improve pest surveillance and management.

9.4 Incidence and seasonal variation of *L.e. pseudobrassicae* and *M. persicae*

Seasonal changes in the tropics, characterized by minimal changes in temperature is seen in the alternation between the rainy seasons and one or two dry seasons within a year (Wernstedt, 1972). Such climatic seasonal changes tend to translate into seasonal activity patterns of insects and often become complex depending on the variety of seasonal patterns in a particular location (Ricklefs, 1975; Wolda, 1988). With the current changes in climate, the dynamics of crop pests are changing drastically, with many pests' variation trends even within one season (Venkataraman, 2013; Skendžić *et al*., 2021). This in turn poses a major challenge to management (Venkataraman, 2013). Chapter five of this thesis assessed the seasonal incidence and variation in the population of *L.e. pseudobrassicae* and *M. persicae* in two agro-ecological zones of Ghana. The relationship between their associated natural enemies and climatic factors was also studied. The findings of this study revealed that both aphids had different seasonal patterns, with multiple population peaks within

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and across seasons, which corroborates with reports by Venkataraman (2013). This also suggests the importance of aphid sampling during each cropping season before application of treatment regimes, to prevent populations from reaching economic thresholds. Since crop yields are largely dependent on pest pressure faced by farmers, and the available control measures (Waterfield and Zilberman, 2012), the changing pest variations may be the reason for calendar spray regimes employed by farmers. Although this study revealed high aphid densities during cropping seasons within the minor rainy and dry seasons, the same cropping seasons between 2019 to 2021 showed different aphid incidence and population variation trends. This confirms the fact that climate change will affect the onset of seasons, which will in turn have an impact on insect seasonal patterns (Wolda, 1988; Danks, 2007). In this light, Walther *et al*. (2002) reported that insects can alter their phenology to adapt to new changes in seasonal climatic conditions or adjust their tolerance to temperature to match local temperature changes.

Temperature is one of the major climatic factors that directly affect pest reproduction, survival, spread and population dynamics as well as their bio-ecological relationships (Bale *et al*., 2002; Stange and Ayres, 2010; Hullé et al., 2010; Skendžić *et al*., 2021). In light of the current changing climatic conditions, monitoring aphid population incidence and abundance is important, as their occurrence may change frequently. Qaisar *et al*. (2014) reported that the variation in weather parameters plays an important role in the growth, development, distribution and population dynamics of aphids. This study recorded a strong relationship between aphid population and temperature further confirming earlier reports (Bale *et al*., 2002; Skendžić *et al*., 2021). This further verifies studies by Rao *et al.* (2013), where temperature was the major factor regulating aphid population of brassica. He further reported a short generation time for aphids during higher

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temperatures, compared to lower ones. Thus, with the changing climatic conditions, it is fundamental to monitor aphid populations, not only at the farm level but at national levels using tools such species distribution models as elucidated in chapter four (4) to map pest hotspots. Nevertheless, climate changes can also have an indirect effect on insect pests by affecting their host or natural enemies (Stange and Ayres, 2010). For example, if natural enemies are more sensitive to temperature changes than aphids (prey), there will be a greater population regulation through predation or parasitism, and vice versa (Berggren *et al*., 2009). However, the shortened developmental time of aphids at high temperatures within the optimum range compared to their predators may narrow the prey vulnerability timeframe for an attack. A similar finding was reported by Virtanen and Neuvonen (1999) with parasitoids. There is also a possibility that because insects in the tropics are currently living within conditions quite close to their thermal optimum, insects may be prone to drastic population decline or extinction if the temperatures go beyond the optimum range (Deutsch *et al*., 2008). In this context, the small populations of *Cheilomenes* sp, *C. carnea*, and *A. varipes* recorded in this study may be partly due to this effect, although subject to further investigation. Earlier studies in the same region where this study was carried out recorded high population densities of *Cheilomenes* sp associated with aphids on cabbage (Fening *et al*., 2014; Forchibe *et al*., 2017), which was not the case in this study.

With the growing concerns on food safety, the use of biological control in pest management programmes provides a great opportunity. Biological control is one of the safest and economically profitable pest management methods (Neuenschwander *et al*., 2003; Naranjo *et al*., 2015). This study identified a vast number of natural enemies associated with aphids on cabbage, which could be explored for biological control. In line with this, several authors have reported successful trials

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with some of these natural enemies as iterated in chapter two of this thesis. For example, field release of the hoverfly larvae, *Episyrphus balteatus* in vegetable fields in China reduced aphid population by over 90% in three days (Youlan *et al.*, 2002). Hassan (1978) reported a significant reduction of *M. persicae* on aubergine, when *C. carnea* larvae were released every 2-5weeks. The predatory gall midge, *A. aphidimyza* reduced *M. persicae* population in greenhouse vegetable cultivation because it reproduces faster than the aphid (Gilkeson and Hill, 1987). The findings of the study showed a positive relationship between both aphids and the hoverfly *P. borbonicus* in all the cropping seasons in both agro-ecological zones, suggesting it could be considered for biological control of aphids in greenhouse vegetable cultivation in Ghana. Field controls can be focused on manipulating habitats around field crops to provide floral food sources for adult hoverflies. Studies on habitat manipulation for pest biological control have been widely reviewed in Wratten *et al.* (2007) and is considered a great option in biological control when classical release methods are inefficient.

9.5 Bionomics of *L.e. pseudobrassicae* and *M. persicae* on three cabbage varieties

Understanding the population growth parameters of *L.e. pseudobrassicae* and *M. persicae* on three cabbage varieties; oxylus, fortune and leader cross, provide evidence to validate the fact that the growth and development of insect pests is affected by the variety or cultivar of the host plant (Kennedy and Abou-Ghadir, 1979; Yue and Liu,2000). The study revealed varied resistance of both aphid species to the three cabbage varieties. The resistance of aphids to brassica plants has been attributed to a wide array of plant characteristics such as colour, palatability, waxiness, trichromes, all of which can be grouped in a broader term as antixenosis (Van Emden, 2017). Antixenosis as resistance to colonization by aphids is often characterized by a high number of

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alates emigrating from the plants. Van Emden (2017) further iterated that, aphids can also experience antibiosis (where, colonized plants negatively affect multiplication of aphids, seen as a decrease in survival, growth and fecundity, and a longer generation time) or tolerance (where the presence of the aphids does not affect the yield of the plant). In this light, the results from this study show that *L.e. pseudobrassicae* and *M. persicae* experienced antibiosis on leader cross and fortune respectively, which recorded low fecundity and survival rates with longer developmental time. The observed reactions to these cabbage varieties could also be attributed to plant chemical composition. Studies by Liu *et al*. (2021) show that high glucosinolate content of cruciferous crops plays a key role in resistance to diseases and pests. Ahmed *et al*. (2019) reported that *M. persicae* had a preference for cabbage cultivars with high nitrogen content. Therefore, it is recommended to further study the chemical composition of these cabbage varieties and how it affects growth and development as well as choice for feeding, to better understand the specific factors responsible for the observed responses to the cabbage varieties.

The results of this study did not concur with the findings of Adenka *et al*. (2021), who under field conditions, reported that fortune was the most susceptible to infestation by *L.e. pseudobrassicae* and *M. persicae*, while oxylus was the most resistant to both aphid species. To account for the disparity in these findings, we hypothesise that under field conditions, several factors affect aphid choices such as inter and intra-specific competition, environmental factors, plant colour, phytochemicals and other biotic factors such as predatory pressure. Unlike in the field study, aphids were enclosed in clipped cages from birth to death and were not allowed to make a choice. High aphid numbers could also be as a result of the influx of alates looking to colonise new plants,

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and not only a matter of host fitness. To affirm this, Yue and Liu (2000) reported that *L. erysimi* landed and fed on green varieties of cabbage, rather than the red.

With the increasing world population and changing climate, breeding for high yielding crops, that are resistant to pest and disease attack is fundamental (Abu *et al*., 2021). The results of this study clarify the importance of host-plant interactions in breeding programmes, as it is widely known that to breed for insect-resistant plants, identification of plant material with resistant genes against the pest is key (Smith and Boyko, 2007). The findings of this study reveal that fortune and leader cross varieties could be explored for resistant genes against *M. persicae* and *L. e. pseudobrassicae* for the development of resistant cabbage lines. Although these findings appear to be promising in the light of host plant resistance, some scientists have argued that because aphids are more restless on resistant varieties, they tend to accelerate the spread of non-persistent viruses (Van Emden, 2017). However, further studies found a negative correlation between non-persistent virus spread and aphids' resistance (Kishaba *et al*., 1992; Chen *et al*., 1997). Host plant resistance shows great potential in the reduction of aphid populations on cabbage, nevertheless, it must be used in combination with or as a component of an integrated pest management strategy.

9.6 Identification and characterization of the disease-causing pathogen on cabbage

Viral diseases are a major threat to sustainable food production around the world, and a menace to global food security (Mumford *et al*., 2016). Coupled with current changes in climate and global exchange of plant materials, the distribution of hosts and vectors have been affected (Anderson *et al*., 2004; Elena *et al*., 2014). Additionally, exchange of plant materials has eased the movement of pathogens and associated vectors into new environments (Jones, 2009). This chapter (Chapter

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7) sought to identify and characterize the pathogen causing a novel necrotic disease on cabbage. It further studied the relationship between associated aphids and disease occurrence. The study identified *Cucumber Mosaic Virus* (CMV) to be associated with the observed disease on cabbage, which is very unlikely, as only a few studies have reported its occurrence on brassica crops (Moreno *et al.*, 2004; Erkan *et al*., 2013; Liu *et al*., 2017a). The CMV strain (CMV-Gh) identified was found to be a pseudo-recombinant, being reported on cabbage in Ghana for the first time, thus validating the fact that the rapid evolution of viruses is one of the major drivers of virus emergence (Jones, 2009; Rubio *et al*., 2020). Furthermore, the findings showed that CMV-Gh could not be confirmed as the causal pathogen of the observed disease following failed transmission experiments to demonstrate Koch's postulates. This led to the following hypothesis for the observed results (i) the CMV strain does not accumulate to high titre but causes severe symptoms; implying there is no correlation between the amount of virus and symptoms (ii) the strain might be very specific/have a narrow host range and infects only cabbage (iii) CMV-Gh might be co-occurring with another disease pathogen which could not be identified using RT-PCR, leading to the severe symptoms observed on plants (iv) the viral strain is in such a way that, when crushed it falls apart, making transmission and characterization difficult, (v) only aphids might be able to transmit the strain using specific carrier proteins and (vi) CMV might be connected or not connected to the disease. However, these hypotheses are subject to further research to understand their possible contributions to the observed disease symptoms. All cabbage seeds used in Ghana are imported from Asia, Europe and the USA (Agriseed Ltd, AGRIMAT Ltd), thus, it is no doubt that the CMV strain identified was a recombination of strains from the USA and Asia. Neegard (1977) reported that CMV can also be transmitted through seeds, which could be one of the means of transmission in this study. This further suggests the role of international trade in the spread of

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plant pests and diseases as reported in Anderson *et al*. (2004), thus, the importance of implementing plant biosecurity and stringent quarantine measures at country borders, to curb spread.

Aphids have been noted as vectors of at least 50% of plant viruses reported around the world (Brunt *et al*., 1996). Their morphology is exquisitely designed for their role as vectors (Ng and Perry, 2004). The cabbage 'necrotic disease' was found to be associated with *L. e. pseudobrassicae* and *M. persicae* in this study. Further analysis revealed a strong positive relationship between aphid occurrence and disease onset, which was expected because earlier speculations suspected both aphids to be vectors of the disease. If CMV is indeed the causal pathogen, it would be interesting to note that *M. persicae* has been reported to be a very efficient transmitter of the virus, and *L. e. pseudobrassicae* a less efficient transmitter (Gildow *et al*., 2008; Tian *et al.*, 2012). However, as indicated earlier on, cabbage plants that showed symptoms of the necrotic disease always had large numbers of *L. e. pseudobrassicae*, and sometimes with *M. persicae* completely absent on the diseased plant. This notwithstanding does not dispute the role of both aphid species in the observed 'necrotic disease' on cabbage.

Despite the massive efforts by plant protection agencies to prevent pest and disease invasion and spread, drivers such as trade and natural dispersal have allowed several pest and diseases invasion (Anderson *et al*., 2004), as is the case of the Fall armyworm with devastating consequences. Informal cross-border trade poses a major risk to pest and disease invasions which continues in regions around the globe unabated (Bebber *et al*., 2014), for example, informal trade of vegetables along the borders of neighbouring countries of Ghana pose a serious threat to the spread of this 'necrotic disease' and/or potent CMV-Gh strain into other countries in this region. Additionally,

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L. e. pseudobrassicae and *M. persicae* have been reported to be associated with cabbage in some neighboring countries (Sæthre *et al*., 2011; Mondédji *et al*., 2021), thus a likelihood of this disease spreading in this region.

Since it is unclear what pathogen is the cause for the observed disease on cabbage, and curing viral-infected plants is not always feasible, control measures should be focused on prevention or using virus-resistant plants. Studies by Adenka *et al*. (2021) showed that the application of neem oil inhibits infestation by aphids, and also prevents the onset of this necrotic disease on cabbage fields. These findings are consistent with a role for *L. e. pseudobrassicae* and *M. persicae* in the transmission of the novel necrotic disease of cabbage. Conclusively, the results of this study propound the need for more collaborative research to find common solutions to this problem. While further research is still ongoing to understand the role of CMV and aphids in the observed necrotic disease, management strategies can be focused on vector (aphid) control.

9.7 Famer perception of aphids as pests of cabbage, and associated damages

With the growing concern about pesticide use, there is oftentimes, a general perception that: farmers are not literate and as such do not understand pest dynamics, approaches to production and pest management. Chapter eight (8) of this thesis discussed farmers' perception of aphids as pest of cabbage and their associated damages. It further outlines farmers' knowledge on cabbage production and management of pests. Contrary to the generally perceived knowledge about farmers, the findings of this study showed that all farmers interviewed had a good knowledge of aphid dynamics; aphid occurrence and seasonal variation, their associated damage and management. The studies also revealed that among the numerous varieties of cabbage grown in

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Ghana, oxylus was the preferred choice, even though, it has been reported as the most susceptible to pest attack (Amoabeng *et al*., 2013). This variety is also called 'farmers choice'. Timbilla and Nyarko (2004) also reported similar findings, with a large percentage of farmers growing oxylus. Chapter six of this thesis also reveals oxylus as the most susceptible cabbage variety among the others used in the study. From this finding, the major question would be: why would farmers choose to grow a more pest susceptible variety of cabbage? There have been varied responses to this question, with some farmers saying that oxylus heads are more compact and heavier, and so fetches more income. Another farmer said, ''varieties like leader cross is resistant but the heads are usually small, and easily goes rotten after harvesting, so I prefer oxylus because when I harvest it takes a longer time to get rotten''. Another farmer said, ''oxylus is well adapted to our Ghanaian environment and is preferred by consumers than the other varieties''. All of these findings encompass indigenous knowledge, which has in the past contributed to major developments in agriculture (Hart and Mouton, 2005). Indigenous knowledge comprises local practices in communities developed from experiences gained over time, and adapted to local culture and environment, and transmitted from one generation to the next (DeWalt, 1994). It has always been relevant in the development of new technologies and adaptive strategies to changing food systems, and in promoting rural development (Kumar, 2010). Oladejo *et al*. (2021) discussed the basics of indigenous knowledge in cabbage production in Nigeria, its relevance in the development of pest management strategies on cabbage. Olaniran *et al*. (2014) also demonstrated the importance of indigenous knowledge in the management of fruit and vegetable pests. These findings provide more evidence, of the importance of indigenous knowledge in pest management and breeding programmes. Thus, crop production can be improved by conducting pest management and breeding programmes that take into cognizance indigenous knowledge.

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Access to food, coupled with the growing world population is of great importance (Verger and Boobis, 2013; Ali *et al*., 2020). Studies have shown that the use of pesticides to control pests can help reduce yield losses, and has contributed greatly to the increase of global food production (Cooper and Dobson, 2007; Damalas, 2009). Thus, pesticide use is often regarded as an economic, and efficient approach to pest management, for improving crop production (Damalas and Eleftherohorinos, 2011; Imoro *et al*., 2019). As expected, this was the case in this study, where farmers relied on pesticides as the exclusive control strategy against aphids on cabbage. Similar findings have been reported on other vegetable crops in Ghana (Ntow *et al*., 2006; Afari-Sefa *et al*., 2015; Wandaat and Kugbe, 2015; Imoro *et al*., 2019; Aniah *et al*., 2021). Furthermore, in instances of low pesticide efficacy, farmers resort to increasing spray frequency, increasing pesticide dosage, or mixing pesticide 'cocktails', which generally has adverse effects on the environment, food safety, non-target organisms and farmers' health. Travisi *et al.* (2006) indicated the risks associated with pesticide use, despite its enormous benefits. A report by FAO (2014), outlines work-related pesticide poisoning of farmers in farms across Zimbabwe, Mexico and India. Bempah *et al*. (2012) and Otchere *et al.* (2020) reported high pesticide residues in cabbages sold on the Ghanaian market. These all point to uncontrolled indiscriminate use of pesticides. These findings, therefore, present evidence to reports that farmers are not literate enough to understand labels and instructions on pesticides (Ntow *et al*., 2006; Owusu-Boateng and Amuzu, 2013; MoFA, 2014; Wumbei *et al*., 2019). This poses a huge environmental and health risk to both farmers and consumers. In addition to indiscriminate pesticide use, the study also showed that most farmers do not use protective clothing during insecticide application, which is often the case among farmers in Ghana (Amoabeng *et al*., 2013; Kwadzo *et al*., 2015; Okoffo *et al*., 2016). High

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numbers of fatalities among agricultural workers associated with pesticide poisoning have been attributed to erroneous perceptions, literacy level, and careless pesticide handling practices among farmers (Ntow *et al*., 2006; Lekei *et al*., 2014; Kwadzo *et al*., 2015; Okoffo *et al*., 2016). These findings suggest that there is a need for farmer educative programmes to address indiscriminate pesticide usage and safety, to prevent a cascade of environmental and health problems. Such training has been shown to reduce the negative impacts of pesticides; for example, studies by Öztaş *et al*. (2018) showed that training and adoption of safety measures, reduced the risk of pesticide-related health hazards.

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CHAPTER 10

10.0 CONCLUSION AND RECOMMENDATIONS

10.1 Conclusion

From this study, the following conclusions can be drawn:

In Ghana, cabbage is mostly grown in the South, and two aphid species occur on cabbage *M. persicae* and *L. e. pseudobrassicae* with the latter being the most widespread and abundant. Their distribution differs significantly across different agro-ecological zones and periods of the year. Both aphids were found to be most abundant in the Deciduous Forest and Coastal Savannah agro-ecological zones and their population growth was found to be dependent on favourable climatic factors. In this study, conditions during July and September; characterized by minimal rainfall and consistent temperature variations between 27.8º to 30.5ºC were most suitable for population build-up of both aphids. The climatic conditions of the Deciduous Forest and Coastal Savannah zones make them favourable for year-round cultivation of cabbage and thus attract more aphid infestation.

The spatial pattern analysis shows that *L. e. pseudobrassicae* and *M. persicae* occur in clusters on cabbage. Random forest species modelling showed that BIO1 (annual mean temperature), BIO3 (isothermality), BIO12 (annual precipitation), BIO16 (average precipitation of the wettest quarter), and BIO19 (average precipitation of the coolest quarter) were the major underlying climatic factors that affected the observed spatial pattern and subsequent distribution/habitat suitability of both aphids. The model used in this study for the habitat suitability of *M. persicae* and *L. e. pseudobrassicae* in Ghana corresponds with present-day distributions except for non-confirmation for sites that have not yet been visited. Nevertheless, the presence of *L. e. pseudobrassicae* is only

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restricted to regions where brassica crops are grown since it is a specialist brassica aphid. The model showed more favourable habitats for both *L.e pseudobrassicae* and *M. persicae* in the South compared to the North of Ghana, which is already noted as the major hotspots for cabbage cultivation.

Lipaphis erysimi pseudobrassicae and *M. persicae* occur on cabbage with different seasonal incidences and population variations. Population peaks across seasons are frequent, suggesting that farmers should perform sampling throughout the year and not just rely on calendar spray regimes. Low aphid population densities recorded within the major season (third cropping season) suggest that this period might be good for the cultivation of cabbage due to low pest intensities associated with rainfall. Aphid population abundance is dependent mainly on temperature and relative humidity, but this might differ depending on the cropping season, as well as the agro-ecological zone. Aphid populations are also affected by natural enemies mainly the syrphid flies, whose population tends to increase with the increase in aphid population. S1, S4 and S2, S5 falls within the minor rainy and dry seasons respectively, characterized by high aphid population densities. Therefore, cabbages produced during this season were prone to high infestation by aphids.

The growth and development of aphids are affected by plant variety. Based on the results of this study it can be concluded that oxylus is the most susceptible variety to both *L. e. pseudobrassicae* and *M. persicae*, while leader cross is resistant to *L. e pseudobrassicae* and fortune to *M. persicae*. Therefore, leader cross and fortune can be used as potential sources of aphid resistance in breeding programmes. These two varieties can also be recommended for use by farmers because using

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resistant plant varieties is economical and reduces the cost of pest control by minimizing continuous pesticide use. Thus, using resistant or less susceptible cabbage varieties is a primary pest management strategy. For studies involving aphid natural enemy trials, oxylus variety can be used for mass rearing of *L. e. pseudobrassicae* and *M. persicae.*

Cucumber Mosaic Virus (CMV-Gh) has been identified to be associated with the observed disease symptoms on cabbage. This was the first report of the Cucumber Mosaic Virus on cabbage in Ghana. Following partial characterization of the genome, the CMV-Gh strain is suggested to be a pseudo-recombinant, arising from the re-assortment of genomic RNAs of indigenous strains and imported CMV strain. Transmission experiments to confirm the pathogen as the disease causal organism following Koch's postulate were unsuccessful. The relationship between aphid incidence and disease onset suggests that aphids play a key role in the disease onset. Findings from the study also suggest that infected plants tend to deter aphids; a tactic used by viruses such as CMV, where CMV-infected plants tend to ward off aphids despite the initial to efficiently spread the pathogen to newer plants.

From the questionnaires administered to farmers, the results showed that they are well versed with aphids as a pest on cabbage, their incidence and seasonal trends which conforms with findings from the incidence and seasonal variation studies in chapter 5. Based on this study, cabbage production is mostly done by males between the age group of 36 - 45 years. Most of the farmers had no formal education, which is reflected in their choice of pesticides, frequency of application and use of protective clothing during pesticide application. A good number of them had agriculture-based training, with more than five years of experience cultivating cabbage which also

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reflected in their understanding of pest behavior, pesticide choice and spray regimes. Additionally, most farmers either mix different pesticides, increase the dosage or increase spray frequency to deal with problems of pesticide inefficacy. There is therefore a need to educate farmers on pest management methods and pesticide use, to promote judicious use, farmer and consumer safety. Farmers grow mainly oxylus variety despite the findings that it is highly susceptible to pest infestation. This suggests that the choice of a particular variety goes beyond pest tolerance to farmer acceptance, yield and consumer preference. Thus, farmers' knowledge must be sought when breeding for new crop varieties.

10.2 Recommendations

From the findings of this study, the following recommendations have been made:

The Northern parts of Ghana should be explored as potential areas for cabbage cultivation, due to less pest intensity recorded. Additionally, the species distribution model developed in this study could be improved upon by incorporating other factors that affect aphid distribution, and further used pest hotspots for timely intervention.

The rainy season should be considered as a more suitable period for cabbage cultivation due to the natural pest suppression associated with rainfall, thus reducing frequent pesticide applications. It is also recommended that leadercross cabbage variety can be used by farmers as a less susceptible variety, as a component of an integrated pest management approach.

Nevertheless, this thesis was limited in several aspects:

Firstly, the survey for aphids' distribution was limited to only nine regions and three districts per region in Ghana. Other districts and regions reported to intensively cultivate cabbage were not

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sampled, for example, the Kwahu district. Additionally, other studies on cabbage in Ghana has reported *B. brevicoryne* as the major aphid on cabbage in the Eastern region (Fening *et al*., 2011, 2014), in the Ashanti region (Baidoo *et al.*, 2012), in the North and Brong Ahafo regions (Osei *et al.*, 2013), and in the Volta region (Ngosong *et al*., 2020). Therefore, before making concrete conclusions on the occurrence of only *L. e pseudobrassicae* and *M. persicae* in Ghana, there is a need for further sampling and identification to be carried out in the country. Secondly, the distribution of *L. e pseudobrassicae* and *M. persicae* across Ghana is limited only to the sites sampled in this study, and to confirm the results of the habitat suitability maps we recommend more sampling and identification. Species distribution modelling is a great tool to understand pest behavioural patterns under changing climatic conditions, and thus, recommended for aphid distribution studies and forecasting.

Studies on aphid bionomics were done under ambient weather conditions and using only three cabbage varieties, thus, it is recommended that trials including more varieties under ambient and field conditions should be carried out to provide additional information on the resistance of cabbage varieties to aphids in Ghana. Furthermore, it would also be relevant to carry out further studies to identify specific factors in these cabbage varieties that influence the growth and development of aphids. Since transmission experiments were unsuccessful, it is recommended that further studies be carried out to understand the role of cucumber mosaic virus and aphids in the disease expression on cabbage.

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APPENDIX

Appendix 1: Surveyed Districts with geo-referenced positions

Location	Latitude	Longitude	Region	AEZ
Ga East	5.689402	-0.20994	Greater Accra	Coastal Savannah
Tema metropolitan	5.638106	-0.01377		
Dzorwulu	5.619875	-0.19285		
Binduri	11.03642	-0.27703	Upper East	Sudan Savannah
Bawku municipal	11.12189	-0.27948		
Bawku West	10.99591	-0.4427		
Sunyani west	7.40385	-2.4683	Brong-Ahafo	Transition
Sunyani municipality	7.336419	-2.3315		
Asutifi south	6.930332	-2.35671		
Suhum municipality	6.102678	-0.48538	Eastern	Deciduous Forest and Coastal Savannah
Akuapem North	6.010377	-0.04927		
New Juaben	6.199228	-0.36417		
Lower Manya	6.009533	-0.04432		
Agona West Municipal	5.518402	-0.69881	Central	Coastal Savannah and Deciduous Forest
Cape Coast metropolitan	5.121389	-1.26833		
Awutu senya East	5.564325	-0.45409		
Kumasi Metropolis	6.684633	-1.55401	Ashanti	Deciduous Forest
Breman Asikuma	7.00093	-1.52166		
Oforikrom	7.04252	-1.92509		
Tamale metropolitan	9.41728	-0.83469	Northern	Guinea Savannah
Sagnarigu	9.442376	-0.90464		
Ketu South	6.126597	1.195946	Volta	Coastal Savannah
South Tongu	5.784232	0.894835		
Keta	6.152568	0.374835		
Techiman municipal	7.51027	-1.91852	Bono East	Deciduous Forest
Kintampo	8.0596	-1.72955		

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Appendix 2: Blast results for aphid identification

Raw sample	Sample Id	Query sequence length	Percentage of identical matches	Closest subject sequence genebank identity	Primer	Species identity	Common name	Country
Green aphid	B1_BA	683	100	MN267876.1	LCO1490	*Lipaphis pseudobrassicae*	turnip aphid	Ghana
Green aphid	B2_BE	688	100	MN267874.1	LCO1490	*Lipaphis pseudobrassicae*	turnip aphid	Ghana
Pink aphid	B3_BA	684	100	MH543312.1	LCO1490	*Myzus persicae*	green peach aphid	Bangladesh
Green aphid	C1_CT	684	100	MN267876.1	LCO1490	*Lipaphis pseudobrassicae*	turnip aphid	Ghana
Green aphid	C60_VT	682	100	MN267874.1	LCO1490	*Lipaphis pseudobrassicae*	turnip aphid	Ghana
Pink aphid	E1_AS	686	99.84	MN232006.1	LCO1490	*Myzus persicae*	green peach aphid	Belarus
Black aphid	E2_AS	680	96.14	LC260610.1	LCO1490	*Alloxysta nr. brevis*	Hyperparasitoid	Japan
Pink aphid	E5_ER	689	99.69	MN232006.1	LCO1490	*Myzus persicae*	green peach aphid	Belarus
Green aphid	E6_KP	685	99.7	MN232006.1	LCO1490	*Myzus persicae*	green peach aphid	Belarus
Green aphid	E7_KYE	683	100	MN267874.1	LCO1490	*Lipaphis pseudobrassicae*	turnip aphid	Ghana
Green aphid	E8_KP	691	100	MN267874.1	LCO1490	*Lipaphis pseudobrassicae*	turnip aphid	Ghana
Pink aphid	E9_KYE	685	99.69	MN232006.1	LCO1490	*Myzus persicae*	green peach aphid	Belarus
Green aphid	G2_AGB	684	99.52	MN232006.1	LCO1490	*Myzus persicae*	green peach aphid	Belarus
Green aphid	G3_DZW	688	100	MN267876.1	LCO1490	*Lipaphis pseudobrassicae*	turnip aphid	Ghana

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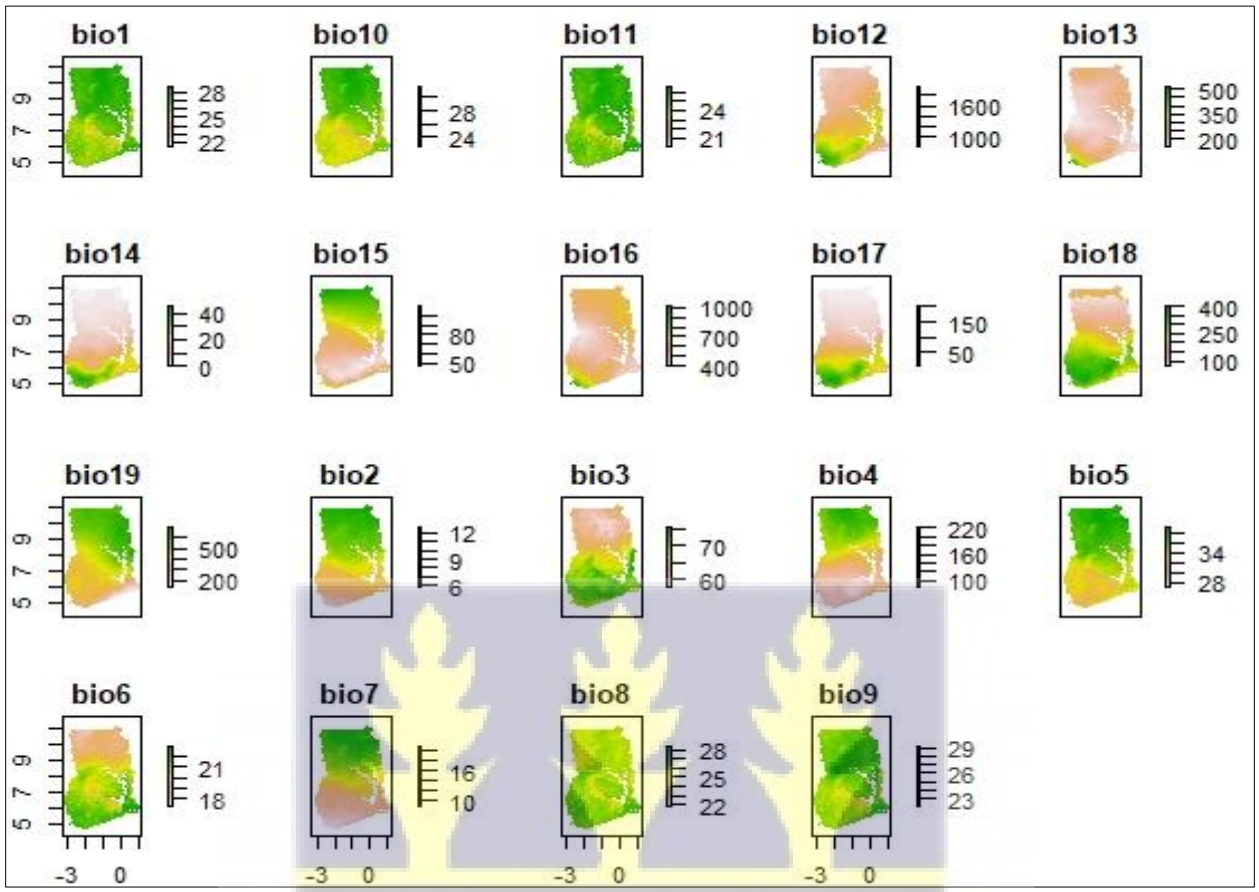
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Raw sample	Sample Id	Query sequence length	Percentage of identical matches	Closest subject sequence genebank identity	Primer	Species identity	Common name	Country
Green aphid	G20_TM	687	99.63	MN267874.1	LCO1490	*Lipaphis pseudobrassicae*	turnip aphid	Ghana
Pink aphid	G21_TM	685	97.48	MN232006.1	LCO1490	*Myzus persicae*	green peach aphid	Belarus
Pink aphid	G24_CT	684	99.69	MN232006.1	LCO1490	*Myzus persicae*	green peach aphid	Belarus
Pink aphid	G25_KMS	685	99.84	MN232006.1	LCO1490	*Myzus persicae*	green peach aphid	Belarus
Pink aphid	G27_BE	687	99.83	KY586056.1	LCO1490	*Myzus persicae*	green peach aphid	India
Green aphid	G28_KMS	688	100	MN267875.1	LCO1490	*Lipaphis pseudobrassicae*	turnip aphid	Ghana
Pink aphid	P10_KP	685	100	MH543312.1	LCO1490	*Myzus persicae*	green peach aphid	Bangladesh
Pink aphid	P14_GGN	684	99.7	MN232006.1	LCO1490	*Myzus persicae*	green peach aphid	Belarus
Pink aphid	P15_SWN	683	100	MH543312.1	LCO1490	*Myzus persicae*	green peach aphid	Bangladesh
Green aphid	P16_GGN	683	99.82	MN267874.1	LCO1490	*Lipaphis pseudobrassicae*	turnip aphid	Ghana
Pink aphid	P17_KPN	685	100	MH543312.1	LCO1490	*Myzus persicae*	green peach aphid	Bangladesh
Green aphid	P18_KPN	687	100	MN267876.1	LCO1490	*Lipaphis pseudobrassicae*	turnip aphid	Ghana
Pink aphid	P20_SGN	683	100	MH543312.1	LCO1490	*Myzus persicae*	green peach aphid	Bangladesh
Green aphid	V1_DNV	683	99.84	MN267874.1	LCO1490	*Lipaphis pseudobrassicae*	turnip aphid	Ghana
Pink aphid	V2-VT	681	100	MH543312.1	LCO1490	*Myzus persicae*	green peach aphid	Bangladesh

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Appendix 3: Bioclimatic variables used in modelling

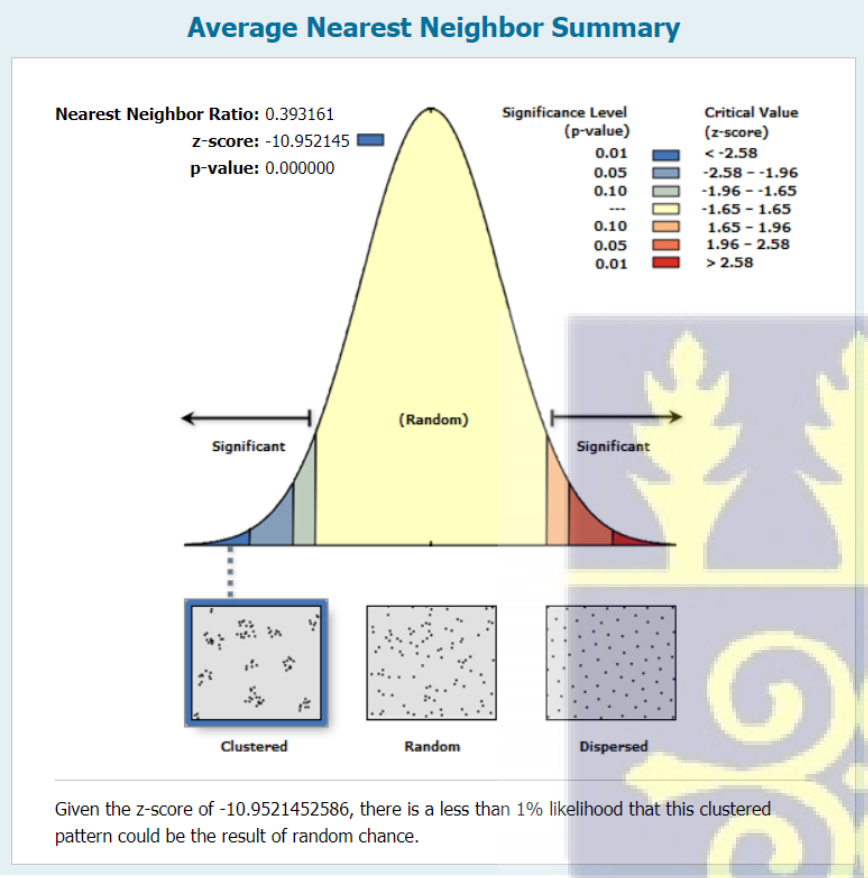


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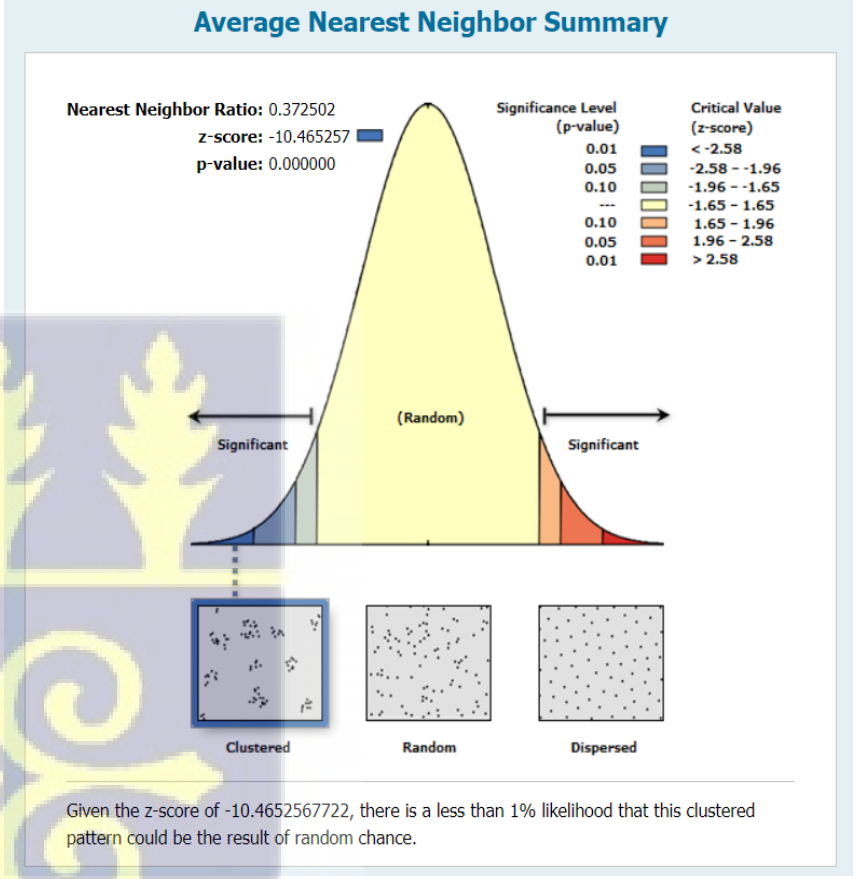
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Appendix 4: Average Nearest Neighbour visualised results for *L.e. pseudobrassicae* points (A) and *M. persicae* points (B)

A



B



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Appendix 5: Primers used to sequence the *Cucumber mosaic virus* genome

Forward primer	**Sequence 5'-3'**	**Reverse primer**	**Sequence 5'-3'**
CMV RNA1 5'	GTTTTATTTACAAGAGC	CMV 1 5 REV	TACTTTCCTCCATACCGATT
CMV1.1F	TTTATTTACAAGAGCGTACG	CMV1.1R	ATACCGTAAGTCATCCCTC
CMV1.2F	ATGTTCATGAYTGGGAGAAT	CMV1.2R	AATTGTTTCTCATAAGCGG
CMV1.3F	CTCAAACGCCTGATG	CMV1.3R	CGAGATGTAACAGATCTT
CMV1.4F	CTGATGAAACTAGTGATGC	CMV1.4R	GTACCCTGAAACTAGCAC
CMV1 3 FWD	AACCGTCTGAAGTCACTAA	CMV RNA1 3'	TGGTCTCCTTTTAGAGAC
CMV RNA2 5'	GTTTATTTACAAGAGC	CMV 2 5 REV	TTCCAAGGTAATCTTCAGCA
CMV2.1F	TTTATTTACAAGAGCGTACG	CMV2.1R	CGAGTCACAAATGGATTG
CMV2.2F	GTTGATAATGCGACTCTG	CMV2.2R	CTAATAGGCATACCAACACC
CMV2.3F	GTCCAGCTCCGATAAC	CMV2.3R	TGGGACTTTTGTGACCTC
CMV2.4F	TGACAAACGTCGAACTC	CMV2.4R	GTCTCCTTTTRGAGGCC
CMV 2 3 FWD	AAGTCACTAAACACATTG	CMV RNA2 3'	TGGTCTCCTTTTAGAG
CMV RNA3 5'	GTAATCTTACCACTGT	CMV 3 5 REV	GTGAATTGTGCTCTAAAGG
CMV3.0F	CGTGTTGTCCGCACATTTGA	CMV3.0R	CTACTGGTACCTTGGAAAGCCA
CMV3.1F	CTTACCACTGTGTGTGTG	CMV3.1R	CGTTAACAAATATTGCGAAG
CMV3.2F	AACAACATTGAATCTTCGC	CMV3.2R	AATCAAATTTCGGCAAAGG
CMVr3.3F	GTCCGTAAAGTTCCTGC	CMVr3.3R	GTACCCTGAAACTAGCAC
CMV 3.4F	TCAAAAGACGATGCG	CMV3.4R	GCCCCCACGAAAG
CMV 3.5F	ACCATCTCCTAGGTTTCTTCGG	CMV 3.5 R	GTCTCCTTTTGGAGGCCC
CMV 3 FWD	AGAACCCTCCCTCCGAT	CMV RNA3 3'	AGGTCTCCTTTTGGAGAC

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Appendix 6: CMV Isolates used in phylogenetic analysis

	Strain	Subgroup	GenBank IDs			Origin
			RNA1	RNA2	RNA3	
1	Fny	IA	D00356	D00355	D10538	United States
2	Leg	IA	D16403	D16406	D16405	Japan
3	Mf	IA	AJ276479	AJ276480	AJ276481	South Korea
4	Y	IA	D12537	D12538	D12499	Japan
5	Bn57	IA	HF 572914	HF 572914	HF 572914	USA
6	Nt9	IB	D28778	D28779	D28780	Taiwan
7	Tfn	IB	Y16924	Y16925	Y16926	Italy
8	Sd	IB	AF071551	D86330	AB008777	China
9	IA	IB	AB042292	AB042293	AB042294	Indonesia
10	Ix	IB	U20220	U20218	U20219	Philippines
11	Vir	IB	HE962478	HE962479	HE962480	Italy
12	Q	II	X02733	X00985	M21464	Australia
13	Ly	II	AF198101	AF198102	AF198103	Australia
15	S	II	Y10884	Y10885	AF063610	South Africa
16	LS	II	AF416899	AF416900	AF127976	United States
17	Trk7	II	AJ007933	AJ007934	AJ007936	Hungary

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Appendix 7: Correlation coefficients for disease severity and aphid abundance

	L. e. pseudobrassicae	*M. persicae*	*Disease severity*
L. e pseudobrassicae	1		
M. persicae	0.401977885	1	
Disease severity	-0.87352502	-0.46773	1

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Appendix 8: Questionnaire on farmers' perception of aphids as pests of cabbage

We are researchers from the University of Ghana currently working on the diversity, biology, distribution and damage impact of aphids on cabbage. This questionnaire is administered to farmers growing cabbage in Southern Ghana (Major cabbage growing sector of Ghana), to understand their perception of aphids as pests of cabbage, their damage and control measures and the impact of this pest on production. Consent must be provided by each farmer before this questionnaire is administered. Information gotten will be used to assess the impact of aphids on the production. Any personal information provided will be treated as confidential. All questions are straightforward requiring very short answers.

NB. ≤ = less than or equal to, ≥ = greater than or equal to and

Thank you for humbly accepting to answer this Questionnaire. < = less than.

Agro ecological zone: …… Region: …… District: ……

Location: …… GPS coordinates: ……

A. Demographic parameters

1. Age: a. (15-25) b. (26-35) c. (36-45) d. (46 and above)
2. Sex: a. □ M b. □ F
3. Do you have any formal education?
   a. □ Yes b. □ No

3.1. If yes, what is the highest education level you have completed?

a. Basic b. Secondary c. Tertiary d. Higher

4. Have you received any training in agriculture? **a. Yes** **b. No**

4.1 If yes, in what aspect (s) were you trained? (Kindly tick all that apply)

a.□ Crop production and protection b.□ Pest Management/IPM c.□ Food safety/Quality

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d.□ Marketing e.□ others (Specify) ……...

B. Knowledge on cabbage production

5. How long have you been growing cabbage?

a. (1-2years) b. (3-5years) c. (5 and above) d. (< one year)

5.1. What varieties of cabbage do you grow? …….

6. How many farms do you have? .....

6.1. What is the size of each farm? ..... **a. (≤ 1 acre) b. (2-5 acres) c. (≥ 5 acres)**

7. Which seasons do you usually grow cabbage? **a. (Major season) b. (Minor season) c. (dry season) d. (a & b) e. (All of the above)**

8. Which season is the best for its cultivation? **(Major season) b. (Minor season) c. (dry season) d. (a & b) e. (All of the above)**

9. Is it a lucrative agribusiness? **a. Yes b. No**

If Yes, explain ……
……

C. Knowledge and perception of aphids and their damage on cabbage

10. Do you know about aphids? **a. Yes b. No**

10.1 If yes can you identify some on the pictorial key provided? **A. Yes b. No**

10.2 How do you locally call it? .....

11. What time of the year do aphids appear on the field? .....

12. What stage of a plant during a growing season does it come? **A. 1week after transplant b. 2 weeks after transplant c. 3weeks after transplant d. Nursery stage**

13. Can you list some symptoms of aphid infestation on cabbage? e.g stunting, yellowing, wilting

……
……

14. Which season has the highest population of aphids? **a. (Major season) b. (Minor season) c. (dry season) d. all of the above**

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15. How much damage does aphids cause? (Rate the damage using the ranges listed) a**. (0-5%) b. (10-20%) c. (30-50%) d. (50% and above)**

15.1. Do you attribute this damage to only aphids or there are other pests associated? **a. Yes b. No**

15.2. Can you list them or identify them from pictorial key provided? **A. Yes b. No**

……

D. Management practices

16. What do you do when you see aphids on your cabbages?

a. Nothing b. Spray insecticide c. Physical removal d. Biological control e. Seek professional advice

16.1 If you apply insecticides, what type do you apply?

a. (synthetic) b. (botanical) c. (biological)

16.2 List the insecticides you usually use against this pest

……
……

16.3. How often do you spray and at what time of the day? **A. Morning B. Afternoon C. Evening D. Anytime**

16.4. Do you wear protective clothing during spraying? **A. Yes B. No**

16.5. Per your experience, are these chemicals/control measures effective? **A. Yes b. No**

If not, what do you do?

a.□ **increase dosage b.□ spray more often c.□ mix pesticides d.□ other…….**

17. Do you combine practices? **A. Yes b. No**

If yes list the practices you employ ……

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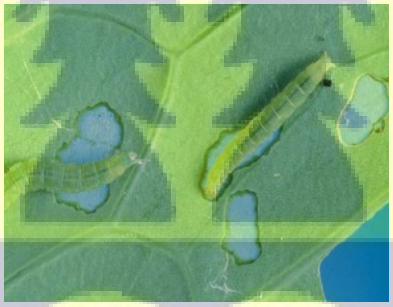
Pictorial key for insect pests on cabbage for farmers

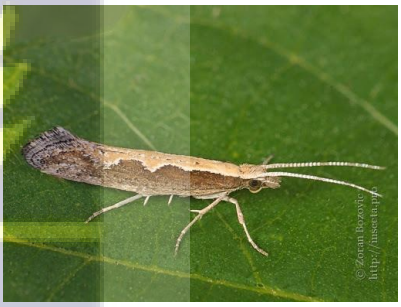




Aphids (*Lipaphis erysimi pseudobrassicae and Myzus persicae*)







Diamond back moth; *Plutella xylostella*







Cabbage webworm; *Hellula undalis*

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Cabbage flea beetle; *Phyllotreta cruciferae.*





Cabbage white butterfly; *Pieris rapae*



Snails

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