Evaluation of ash and soil for control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) on maize

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Abstract

Fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) emerged as one of the most important global pests of maize in recent years. The crops that are most infested in Africa are maize and sorghum. In its native area of the Neotropics in Central and South America, *S. frugiperda* is largely controlled by means of Bt maize and the application of chemical insecticides. The arrival of this invasive pest species threatens food security in Africa. Unfamiliarity with this pest resulted in large scale use of insecticides in many countries, especially by small holder farmers, which, in the past did not often apply insecticides for control of maize pests. The use of insecticides is in most cases not affordable to small holder farmers. Alternative control methods, for example, biopesticides are also used for *S. frugiperda* control. These methods are more appropriate for smallholder farmers in developing countries because they are readily accessible and not expensive. Soap solutions, ash, sand and soil, applied into whorls of maize plants to control *S. frugiperda* larvae are other alternative methods used by smallholder farmers. The efficacy of many of these natural local innovations have not been determined under controlled conditions, and variable results have been reported from farmers' fields. The aim of this study was therefore, to determine the effect of the application of soil and wood ash into the whorls of maize plants on the mortality and movement behaviour (ballooning/escape) of fall armyworm (FAW) larvae. Bioassays were also conducted in which the preferences of 1st- and 3rd-instar larvae for whorl leaf tissue treated with ash and soil were determined. Pilot experiments, followed by 11 laboratory and field trials were conducted. In these experiments, ash was applied as either a preventative treatment (before larvae were inoculated onto plants) for 1st-instar larvae, or a curative treatment (after larvae were inoculated onto plants) for 1st-, 3rd- and 5th-instar larvae. The efficacy of application of wet ash was also compared to that of a dry ash application. The incidence of ballooning of 1st-, 3rd- and 5th-instar larvae was low (ranged from 5 to 21.17%) and did not differ significantly between treatments. Curative application of dry ash against 1st-instar larvae resulted in significantly lower rates of ballooning compared to other treatments. Significantly higher levels of mortality of 1st-instar larvae were recorded inside plant whorls that were curatively treated with wet (65.6%) and dry ash (67.5%), compared to the control treatment (22.0%). This was however not the case with 3rd-instar larvae under laboratory conditions. In preference tests, significantly higher numbers of 1st- and 3rd-instar larvae preferred to settle on untreated leaf tissue compared to the wet ash, dry ash and soil treatments, indicating that the presence of these compounds had a repellent effect. Variable results were recorded in the field trials. The application of ash and soil into plant whorls as curative treatments, resulted in significant reductions in the survival of 3rd-instar larvae in field trials. Significantly lower levels of survival of 3rd-instar larvae were recorded with the dry ash (21.4%), soil (25%) and wet ash (33.9%) treatments, compared to the control (69.6%). However, neither preventative nor curative applications against 1st- instar larvae resulted in significant mortality in field experiments. Leaf damage scores (1-9 rating scale) made 5 days after inoculation of plants with neonate larvae, differed significantly between treatments (range: 3.2 – 4.1). It can be concluded that the use of ash and soil treatments by small holder farmers in Africa and beyond has a potential to contribute to integrated pest management (IPM) against *S. frugiperda* larvae. However, these local innovations are not always effective in controlling *S. frugiperda* larvae and the level of efficacy of control varies a lot as observed in this study. Adoption of local innovations to manage this pest is fundamental to the implementation of IPM. Long-term management of *S. frugiperda* should be based on IPM principles which include the use of local innovations, host plant resistance, cultural control practices and minimizing the application of chemical pesticides to protect natural enemies.

Key words: alternative control methods, fall armyworm, IPM, smallholder farmers.

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Chapter 1

Introduction and literature review

1.1 Introduction

Maize (*Zea mays*) is one of the most important cereal crops globally (Badu-Apraku & Fakorede 2017, Silva *et al.* 2015). Due to its high nutritional value, it is the most cultivated cereal crop across the globe followed by rice and wheat (Kammo *et al.* 2019). In sub-Saharan Africa (SSA), maize is an essential staple crop (Banson *et al.* 2020) with approximately, 300 million African people relying on it as their staple food (Badu-Apraku & Fakorede 2017, Nyaligwa *et al.* 2017). Therefore, it is of critical importance to food security (Badu-Apraku & Fakorede 2017, Nyaligwa *et al.* 2017). Maize is planted on 25 million hectares in SSA, mainly by smallholder farmers and the top producers of maize in Africa are Nigeria, Tanzania, South Africa (9^{th} in the world) and Ethiopia (Badu-Apraku & Fakorede 2017).

Despite the major significance of maize in SSA, yield levels remain low compared to the global mean of 4.5 t ha^{-1} (Nyaligwa *et al.* 2017). These low yields are ascribed to several abiotic and biotic constraints (Badu-Apraku & Fakorede 2017, Silva *et al.* 2015). Biotic constraints include insect pests such as the fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) and maize stem borers (Silva *et al.* 2015). Important weed species that occur in maize fields include cogon grass (*Imperata cylindrica*) and parasitic weeds such as *Striga* spp. Important plant diseases include ear rot, downy mildew, grey leaf spot and maize lethal necrosis disease (Badu-Apraku & Fakorede 2017). Insect pests are a huge problem in all cropping systems worldwide, however, their impacts in SSA are much higher as a result of poverty, lack of knowledge and poor agricultural infrastructure without access to effective crop protection knowledge and/or resources (Grzywacz *et al.* 2014)

FAW is a polyphagous lepidopteran pest with a wide-host range as it feeds on 353 plant species belonging to different families (Montezano *et al.* 2018). However, it mostly feeds on plants in the Poaceae family (Bateman *et al.* 2018). FAW causes significant damage to economically important grasses such as maize, rice, sorghum, sugarcane and may also damage some vegetable crops

(Banson *et al.* 2020). It is native to North and South America (Nagoshi *et al.* 2020) where it is also a serious pest of maize (Banson *et al.* 2020, Silva *et al.* 2015). FAW larvae feeds on both leaves and ears of plants (Kammo *et al.* 2019, Van den Berg *et al.* 2021). The first report of FAW in Africa was in West Africa during 2016 (Goergen *et al.* 2016). By the end of 2017 it spread to more than 30 countries in tropical and southern Africa (Bateman *et al.* 2018). A survey of the population structure of FAW in Africa showed that this pest is under a process of becoming established on the continent, however, the distribution of permanent populations and regional migratory patterns are yet to be determined (Nagoshi *et al.* 2022).

It is suggested that FAW was accidentally introduced into Africa, possibly as stowaways on commercial aircraft, either in cargo containers or airplane holds, after which it spread through wind dispersal (Cock *et al.* 2017). Consequently, the production of maize in Africa faces serious threats, particularly in smallholder farming systems (Shaiba *et al.* 2019, Banson *et al.* 2020). It has been estimated that tens of millions of smallholder farmers in Africa are highly affected as host plants of FAW include maize, millet, sorghum and teff which are staple crops (Bateman *et al.* 2018). Without effective control, the total annual production losses in 12 maize producing countries in Africa were estimated to be 8.5 to 21 million tons to the value of 250 to 630 million US dollars and this further contributes to poverty and food insecurity in Africa (Banson *et al.* 2020).

Maize is susceptible to FAW attack at all stages of the plant's development cycle, from emergence to tasselling and silking (Lima *et al.* 2010). FAW is a foliage and ear feeder, and severe infestations can lead to 100% yield losses in cereal crops (Phambala *et al.* 2020). Young larvae feed on the developing leaves of maize inside the funnel (Bateman *et al.* 2018, Kammo *et al.* 2019). If infestation occurs early in the season, larval feeding results in dead heart by killing the growing point of maize seedlings (Bateman *et al.* 2018, Kammo *et al.* 2019, Sisay *et al.* 2019b) which prevents the formation of ears. During the day, young larvae hide inside the funnel and come out at night to feed on the leaves and mature larvae also remain inside the funnel (Bateman *et al.* 2018), however, if plants are older, larvae feed on maize ears (Kammo *et al.* 2019, Sisay *et al.* 2019b). FAW damage to ears of maize plants damages developing kernels and exposes the ears to infections by microorganisms and fungi that produce mycotoxins (Lima *et al.* 2010). This damage reduces the quality and quantity of yield (Sisay *et al.* 2019b) and furthermore, the cryptic feeding behaviour protects them from natural enemies (Bateman *et al.* 2018, Kammo *et al.* 2019).

Rapid population increase of FAW occurs when climatic conditions are favourable, as this increases the pest's reproduction ability (Roel *et al.* 2010). FAW has remarkable dispersal abilities and can migrate and reproduce quickly. It is one of the most challenging insect pests to control in Africa because it has multiple generations as well as the capability to migrate long distances and feed on various host plants (Sisay *et al.* 2019b).

The cost of management strategies presents serious economic implications (Banson *et al.* 2020) especially for resource-poor farmers (smallholder farmers) who cannot afford to purchase synthetic insecticides (Grzywacz *et al.* 2014, Kayange *et al.* 2019, Stevenson & Belmain 2017, Tembo *et al.* 2018). Furthermore, synthetic insecticides are not ideal for smallholder farmers due to their toxicity, and, since smallholder farmers lack appropriate personal protective equipment (PPE), pesticide use exposes them to the health hazards associated with these products (Bateman *et al.* 2018). In addition, synthetic insecticides used for FAW control often seem to be ineffective (Roel *et al.* 2010, Silva *al.* 2015) because of poor application practices and misuse that leads to resistance evolution (Kammo *et al.* 2019, Phambala *et al.* 2020, Stevenson & Belmain 2017). Most of the cheapest and widely used pesticides for control of FAW in Africa belong to the mode of action classes to which the evolution of resistance has been reported in the Americas (Day *et al.* 2017, Kammo *et al.* 2019, Phambala *et al.* 2020).

Fall armyworm is difficult to control (Matova *et al.* 2020) and poor levels of control are often reported (Van den Berg *et al.* 2021). The cryptic feeding behaviour of FAW (Van den Berg *et al.* 2021) makes it difficult for insecticide spray applications to reach the target area where larvae feed deep within the plant whorls (Van den Berg & Viljoen 2007, Maluleke 2020, Yan *et al.* 2022), especially when contact insecticides are used (Bateman *et al.* 2018, Kammo *et al.* 2019). Therefore, pesticide application methods must be improved to ensure that the applied pesticidal product reaches the target pest (Pimentel 1995). Application of granular formulations into plant whorls have been reported to be highly effective since it ensures the presence of insecticides very close to where larvae feed (Pitre 1986). Soap solutions, ash, sand and soil are alternative methods used by smallholder farmers for controlling several pest species, including FAW (Shaiba *et al.* 2018). Application of these alternative local innovations, which are all applied into plant whorls where larvae feed, could provide tools for smallholder farmers to control of FAW, provided that they are effective.

The need exists to promote low-risk, affordable and effective alternative pest control methods such as pesticidal plants that are applicable to smallholder farmers with less technical knowledge (Belmain *et al.* 2012, Grzywacz *et al.* 2014, Maredia *et al.* 1992). In contrast to synthetic insecticides pesticidal plants reduces the non-target effects (Benelli *et al.* 2017, Saleem *et al.* 2018) on beneficial arthropods at higher trophic levels (Tembo *et al.* 2018). Using pesticidal plants have proved to be more sustainable and suitable for smallholder farmers in developing countries (Kayange *et al.* 2019, Phambala *et al.* 2020, Tembo *et al.* 2018).

Many plant species have pesticidal properties and extracts of these plants can serve as effective alternatives to synthetic insecticides for pest management by smallholder farmers (Grzywacz *et al.* 2014, Mkenda *et al.* 2014). Biopesticides such as neem, *Azadirachta indica,* and fish bean (*Tephrosia vogelii*) extracts are getting more attention as they are eco-friendly (Benelli *et al.* 2017, Saleem *et al.* 2018) and have a low cost of production (Benelli *et al.* 2017, Kammo *et al.* 2019, Maredia *et al.* 1992, Phambala *et al.* 2020). Furthermore, these alternative methods are appropriate for smallholder farmers because they are readily accessible and not expensive (Kammo *et al.* 2019, Maredia *et al.* 1992, Phambala *et al.* 2020). The promotion of pesticidal plants would be a huge benefit to smallholder farmers since it requires less processing (Grzywacz *et al.* 2014).

Smallholder farmers in Africa also use soil or sand (Shaiba *et al.* 2019) mixed with ash/lime (Day *et al.* 2017), as well as botanicals such as neem and *T. vogelii* (Belmain *et al.* 2012) and put these materials into whorls of maize to control FAW larvae. The use of botanicals with insecticidal properties is not a new approach to insect control as these plants were commonly employed prior to the development of synthetic insecticides (Lima *et al.* 2010, Roel *et al.* 2010). Pesticidal plants can be locally propagated and self-harvested, which is one of the greatest advantages since it limits distribution constraints, especially in remote areas (Stevenson & Belmain 2017). Integrated pest management, which involves the use of different control methods in conjunction, is the preferable approach for managing FAW (Day *et al.* 2017). In Latin America, where smallholder farming systems resemble African farming systems, IPM is mostly used for FAW management (Day *et al.* 2017).

1.2 Literature review

Various products such as diatomaceous earth, granular insecticides and biopesticides are used by smallholder farmers to control FAW. Application of soap solutions, ash, sand and soil are other alternative methods used by smallholder farmers for controlling several pest species and have been used for FAW control as well. These methods and materials which can be used by smallholder farmers to control FAW and other pests are reviewed below.

1.2.1 The use of botanicals/pesticidal plants against FAW and other insect pests

1.2.1.1 Efficacy of neem tree extracts against insect pests

Neem is a multipurpose tree from the Meliaceae family (Saleem *et al.* 2018) and is native to North-East India and Burma (Benelli *et al.* 2017). It is widely distributed in Asia and Africa (Maredia *et al.* 1992). Neem is widely used because of its insecticidal properties (Benelli *et al.* 2017). It has a potential of controlling numerous insect pests including FAW (Giongo *et al.* 2016). The ability of neem to control insect pests has been confirmed on over 430 species (Roel *et al.* 2010). The efficacy of neem oils and extracts on insect pests is ascribed to the occurrence of large amounts of limonoids, with azadirachtin as the most complex and strongest compound (Giongo *et al.* 2016). Azadirachtin has a selective activity against many insect pests (Maredia *et al.* 1992). Neem tree leaf, seed, bark, oil and flowers contain compounds with multiple pesticidal activities (Chaudhary *et al.* 2017, Lima *et al.* 2010). Neem tree extracts are also effective against FAW larvae (Cen-Pacheco *et al.* 2020, Saleem *et al.* 2018, Viana & Ribeiro 2010), and can be used efficiently to protect maize from FAW damage (Maredia *et al.* 1992, Viana & Ribeiro 2010).

Neem extracts are effective even in low concentrations (Benelli *et al.* 2017, Roel *et al.* 2010) and plants contain biological compounds with the ability to delaying embryo development at different stages of growth (Kammo *et al.* 2019, Roel *et al.* 2010). Furthermore, neem has repellent and antifeedant properties (Benelli *et al.* 2017, Maredia *et al.* 1992, Saleem *et al.* 2018, Shaiba *et al.* 2019, Silva *et al.* 2015) that retards postembryonic development (Kammo *et al.* 2019) and escalates mortality rates (Phambala *et al.* 2020, Silva *et al.* 2015, Sisay *et al.* 2019a) between moults (Choongo *et al.* 2018). Neem is also known to cause eggs to become sterile and induce sterility (Kammo *et al.* 2019, Roel *et al.* 2010) in insects as it prevents oviposition in females and interferes with the production of sperms in males (Chaudhary *et al.* 2017). Neem can be used in various applications such as neem oil (Kammo *et al.* 2019, Roel *et al.* 2010), neem seed cake (Silva *et al.* 2015), neem seed (Chaudhary *et al.* 2017) neem leaves (Viana & Ribeiro 2010) or neem bark (Shaiba *et al.* 2019), and all these products are reported to be effective.

Neem extracts are not toxic to mammals, birds, fish and humans and it is also used to make herbal medicines, toothpaste, soaps and detergents (Maredia *et al.* 1992, Sisay *et al.* 2019a) which makes it an ideal biopesticide (Chaudhary et al. 2017). The likelihood of resistance evolution against neem products is low because of its multiple modes of action in insects (Chaudhary *et al.* 2017) and complexity of its active compounds (Mkenda *et al.* 2014, Roel *et al.* 2010). However, one concern regarding the use of neem extracts is photodegradation, as azadirachtin breaks down when exposed to sunlight, leading to a reduction in its efficacy over time (Giongo *et al.* 2016).

a) Neem leaves

Neem leaves have pesticidal have repellent and insecticidal properties and, as a result, has been used for centuries against stored-product insect pests ((Silva *et al.* 2015, Chaudhary *et al.* 2017). Furthermore, neem leaves have strong nematicidal, miticidal and fungicidal properties (Lima *et al.* 2010, Saleem *et al.* 2018). The aqueous extract of neem leaves adversely affects FAW larvae by hindering/reducing their development (Roel *et al.* 2010). Viana and Ribeiro (2010) recorded significant reduction on FAW larval development using a neem leaf aqueous extract at a concentration of 500 ppm. Furthermore, neem leaf aqueous extract reduced larval feeding damage to maize plants. Viana and Ribeiro (2010) evaluated different types of neem leaf aqueous extracts (dry ground, green ground and green crushed) and observed no significant difference on maize damage between different extracts. The application times (morning and/or afternoon) also showed no significant difference in controlling FAW larvae, indicating that application time does not affect the efficacy of neem leaf extracts.

Neem leaf extracts enhanced the shelf life of mungbean grain by protecting it against pulse beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae) (Ahmad *et al.* 2015). A neem leaf dose of 1.5 mg/100 g grain resulted in a significant reduction in oviposition and increased adult mortality by 62%, and this indicates that it can be an effective bioactive repellent compound for post-harvest grain storage (Ahmad *et al.* 2015). A concentration rate of 0.25% neem leaf aqueous extract was reported to be effective in reducing FAW infestations and causing high levels of mortality (Silva *et al.* 2015). However, mortality rate is usually low during the first days and increases as the number of days that an insect is exposed to a treatment increases. This indicates that neem extracts require a certain period of time to show effects on larvae (Silva *et al.* 2015, Viana & Prates 2003). Repellent and antifeedant effects of neem leaves were observed to reduce infestation of plants by aphids when applied as an additional component of organic fertilizer that was enriched with neem leaf powder and wood ash (Chaudhary *et al.* 2017).

Lima *et al.* (2010) found that neem leaf extracts increased the grain yield of maize and produced best results for husked green ear mass. In addition, there was greater yield of the number and total weight of marketable ears with husks in plots that were sprayed with neem extract. Soil microorganisms and plant growth-promoting rhizobacteria were improved in soil that was treated with 0.1 and 0.4 g/ml of neem leaf extract (Sarawaneeyaruk *et al.* 2015). Neem leaf powder applied together with neem derived insecticides resulted in a significant reduction in the number of eggs that hatched and survival of *Spodoptera exigua* (Lepidoptera: Noctuidae) larvae (Greenberg *et al.* 2005). Aqueous extracts of neem leaves reduced the population density of *Megalurothrips sjostedti* (Thysanoptera: Thripidae) and increased yield in cowpeas under field trials (Ivbijaro 1990, Tanzubil 1991). Phambala *et al.* (2020) recorded 60% mortality of FAW larvae at a concentration of 10% and also observed that neem leaf extracts showed deterrence effects towards FAW larvae, hence reducing crop damage. The antifeedant properties of neem extracts are well recognized for many lepidopteran pests (Phambala *et al.* 2020).

b) Neem oil

Neem seed oil is the most utilized product of the neem tree (Benelli *et al.* 2017). Neem seed contains 40% of oil (Chaudhary *et al.* 2017) and azadirachtin is the main active ingredient responsible for its insecticidal activity (Chaudhary *et al.* 2017, Lima *et al.* 2010). Neem oil is more efficient against soft-bodied insects and mites and it is efficient in controlling many insect pests including FAW, tobacco armyworm (*Spodoptera litura* (Lepidoptera: Noctuidae)), cotton bollworm (*Helicoverpa armigera* (Lepidoptera: Noctuidae)) (Chaudhary *et al.* 2017) and stem borers such as *Busseola fusca* (Lepidoptera: Noctuidae) and *Sesamia calamistis* (Lepidoptera: Noctuidae) (Kammo *et al.* 2019). Shaiba *et al.* (2019) reported that neem oil sprays were effective in reducing FAW damage in Ghana. In a study conducted by Kammo *et al.* (2019) in Cameroon, neem oil application showed highest efficacy in reducing the incidence of dead hearts compared to insecticide treatments which included cypermethrin and lambda-cyhalothrin. Furthermore, neem oil was the most effective treatment and resulted in a 61% reduction in severity of damage to maize leaves. In the latter study, the incidence of damage by both FAW and stem borers was reduced by 81%.

Roel *et al.* (2010) recorded 100% mortality of FAW larvae in bioassays in which neem oil was applied at a concentration rate of 0.4% added into artificial diet and also observed longer development times of larvae at neem oil concentrations of 0.006 and 0.05%. Shaiba *et al.* (2019) suggested that spraying neem oil-soap aqueous solutions directly into maize whorls could be an effective preventative measure for FAW control. Neem oil induces sterility and act as an antifeedant, larvicidal, ovicidal, insect growth regulator and repellent against insect pests (Benelli *et al.* 2017, Chaudhary *et al.* 2017, Saleem *et al.* 2018). The antifeedant and sterilant effects of neem may contribute to crop protection through the reduction of pest populations without having detrimental effects on non-target insect populations (Chaudhary *et al.* 2017). During the pupal stage of insects, neem oil causes lethal toxicity which results in many morphological deformations including deformed adults (Chaudhary *et al.* 2017, Lima *et al.* 2010), incomplete ecdysis and blocked moulting (Kammo *et al.* 2019, Roel *et al.* 2010, Saleem *et al.* 2018). This then prevents formation of adults (Chaudhary *et al.* 2017).

Giongo *et al.* (2016) reported that commercial neem oil can reduce the weight of FAW larvae and cause phagodeterrency. A concentration of 0.05% neem oil reduced pupal weight of FAW and produced smaller adults and neem oil application at concentrations of 0.006, 0.05 and 0.4% affected the guts of FAW larvae through loss of lining, undulations and degradation of the peritrophic matrix, and necrosis in the midgut epithelium (Roel *et al.* 2010). Neem oil applied at a rate of 5 and 10 ml/kg maize grain reduced the survival of maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae) and larger grain borer, *Prostephanus truncatus* (Coleoptera: Bostrichidae) (Roel *et al.* 2010). Babendreier *et al.* (2020) tested two neem-based products in Ghana i.e., Grow-Safe neem oil and Ozoneem oil. Grow-Safe neem oil mixed with liquid soap at 0.67% and applied at 0.17% (low dose) and 0.33% (normal dose), provided effective protection against FAW. However, no significant impacts were observed on the number of larvae, which indicated that the active ingredient of neem, azadirachtin, acted mostly as an antifeeding agent in this study (Babendreier *et al.* 2020).

c) Neem seed

Neem seed is an effective insect growth regulator as it has significant behavioural and physiological effects on insects (Maredia *et al.* 1992, Saleem *et al.* 2018, Silva *et al.* 2015). Derivatives from neem seed have been used for centuries by Indian farmers to protect agricultural crops from pest attack (Maredia *et al.* 1992). Maredia *et al.* (1992) recorded mortality rates of up to 100% in FAW and corn earworm, *Helicoverpa zea* (Lepidoptera: Noctuidae) after 14 days. Mikolajczak *et al.* (1989) also recorded 100% mortality of FAW larvae after application of neem seed oil at a concentration rate of 16 ppm. Maredia *et al.* (1992) and Roel *et al.* (2010) found that neem seed powder and neem oil impacted negatively on the weight of FAW larvae. Maredia *et al.* (1992) and Mikolajczak *et al.* (1989) also recorded a significant reduction on the weight of FAW larvae after application of neem seed powder.

Mikolajczak *et al.* (1989) recorded extended pupation periods as a result of neem seed extracts incorporated into FAW artificial diet. This long development time might be beneficial in the management of FAW since it may reduce the number of FAW generations per season. Smallholder farmers can sustainably utilize neem extracts as a pest control against FAW and stem borers to improve maize production (Kammo *et al.* 2019). Singh (2003) investigated anti-fecundity effects of neem seed on the development of oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae) and melon fly, *Bactrocera cucurbitae* (Diptera: Tephritidae) and showed that neem seed crude extract had greater effects on fertility and fecundity when compared to aqueous neem seed extract. The efficacy of neem extracts shows that neem can be an affordable, safe and renewable alternative to synthetic insecticides (Singh 2003).

Saleem *et al.* (2018) reported that neem seed extracts of 10 ppm have, through their endocrine systems, a direct toxicity on cells and tissues of *H. armigera* and *S. litura.* Neem seed aqueous extracts showed lethal effects on *Coleomegilla maculata* (Coleoptera: Coccinellidae) (Roger *et al.* 1995). Zebitz (1984) observed prolonged larval development time of larvae of *Aedes aegypti* (Diptera: Culicidae) after application of neem seed extracts into water. Aqueous neem seed extracts protected cassava and melon plants or cassava tubers against termite damage (Umeh & Ivbijaro 1999). Application of neem seed oil was reported to reduce the number of emerging *S. zeamais* larvae from stored grain (Nukenine *et al.* 2011). Sisay *et al.* (2019a) recorded 98% FAW larval mortality, 72 hours after the application of neem seed extracts in laboratory bioassays and in field trials, neem extract killed all larvae, hence protecting maize from FAW damage by reducing leaf injury/damage.

d) Neem seed cake

Neem seed cake is the product which is obtained after neem oil is extracted from seeds. In agriculture, it can also be applied as a pesticide or natural fertilizer (Chaudhary *et al.* 2017). Studies conducted in India and the Philippines, reported that neem seed cake was effective in reducing loss of nitrogen in rice fields (Maredia *et al.* 1992). It is also utilized as a feed supplement for livestock, cattle and poultry. Williams and Mansingh (1996) observed effective control with neem seed cake extracts evaluated against *Bemisia tabaci* (Hemiptera: Aleyrodidae), *Amblyomma cajennense* (Parasitiformes: Ixodidae), *Plutella xylostella* (Lepidoptera: Plutellidae), *Boophilus microplus* (Parasitiformes: Ixodidae), and *Acarina* spp. (Acari).

Silva *et al.* (2015) reported that neem seed cake aqueous extract at a concentration of 0.13% caused high levels of mortality in FAW larvae, in contrast to neem leaf aqueous extracts. This higher susceptibility of FAW larvae to neem seed cake is ascribed to higher content of azadirachtin in the seed cake of neem as it comprises 90% of azadirachtin (Silva *et al.* 2015). These findings show that only small amounts of neem extracts are required to kill FAW larvae. Both neem seed cake and leaf extracts delay the development of FAW larvae. Neem-based extracts disrupt cellular processes and the physiology of ecdysis, which then leads to mortality of insects. Silva *et al.* (2015) also found extracts of neem seed cake to be highly effective in controlling Hemiptera pests when applied into the soil and taken up by plant roots.

e) Neem bark

The pesticidal activity of neem bark is lower than that of neem seed and leaves. Therefore, its use as a bio-insecticide against insect pests is limited. Furthermore, neem bark possesses allelopathic properties when incorporated into soil to control insect pests and it has an adverse effect on germination and growth of many crop species (Chaudhary *et al.* 2017).

1.2.1.2 Efficacy of *Tephrosia vogelii* tree extracts against insect pests

Extracts of *Tephrosia vogelii* (Fabaceae) are known to have deterrent effects and insecticidal properties (Grzywacz *et al.* 2014, Mkenda *et al.* 2014), and have been used for many years as a pest control tool in Africa (Belmain *et al.* 2012). *Tephrosia vogelii* is widely used as a natural, organic pesticide on crops (Choongo *et al.* 2018) and smallholder farmers in SSA use leaf extracts of *T. vogelii* as an insecticide (Harrison *et al.* 2019). Tembo *et al.* (2018) reported that application of *T. vogelii* leaf extracts significantly reduced the abundance of aphids, bean foliage beetles and flower beetles on cowpea and beans, without any effect on the abundance of beneficial species (lady beetles, spiders and hoverflies). Just like the neem tree, active compounds found in *T. vogelii* leaves also have acaricidal, ovicidal and ichthyotoxic effects and could be responsible for stomach poison to insects (Kayange *et al.* 2019). Choongo *et al.* (2018) reported that *T. vogelii* leaf extracts have contact toxicity that has negative effects on the cuticle of many insect pests during the larval stage. Furthermore, a lower risk of resistance evolution exists when *T. vogelii* extracts are used because of its numerous bioactive compounds with low persistence (Tembo *et al.* 2018).

Tephrosia vogelii comprises of two chemotypes, referred to as chemotype 1 and chemotype 2. Chemotype 1 has rotenoids that are responsible for its insecticidal activity, while chemotype 2 has no rotenoids, and therefore, should not be promoted for pest control (Belmain *et al.* 2012). Choongo *et al.* (2018) also confirmed that the presence of rotenoids is responsible for the insecticidal activity of *T. vogelii*. Stevenson and Belmain (2017) reported that *T. vogelii* is widely used in East Africa for protecting stored grains against beetles. Belmain *et al.* (2012) evaluated the efficacy of these two chemotypes on Bruchidae beetles that infest cowpeas and reported that chemotype 1, applied at a dose of 0.55% (w/w), was highly effective (80% mortality). This indicates that *T. vogelii* can be used effectively even at low concentrations, however, aqueous leaf extracts were less effective compared to leaf powder when applied against bruchids. Belmain *et al.* (2012) also observed that extracts of *T. vogelii* deterred oviposition and reduced the numbers of eggs laid by bruchids and that no adults emerged even at very low concentrations of extracts.

Choongo *et al.* (2018) reported that rotenoid compounds of *T. vogelii* compromised the respiratory system of African armyworm, *Spodoptera exempta* (Walker) (Lepidoptera: Noctuidae), which resulted in death of larvae. The larval stage of *S. exempta* is vulnerable to *T. vogelii* leaf extracts as it inhibits and disrupts moulting. Application of *T. vogelii* leaf extracts for *S. exempta* control under field conditions also reduced feeding damage to maize with a resulting reduced loss of yield (Choongo *et al.* 2018).

Kayange *et al.* (2019) investigated the efficacy of *T. vogelii* against black bean aphid, *Aphis fabae* (Hemiptera: Aphididae) and showed that leaf extracts were effective in reducing infestations of black bean aphid. This reduction was attributed to the direct chemical contact and repellent effects of *T. vogelii* since bean aphids have low mobility. The latter study concluded that T*. vogelii* leaf extracts applied at low concentrations are effective in reducing bean aphid numbers to below damaging levels. Efficacy of *T. vogelii* has been also observed against the pod borer, *Maruca vitrata* (Lepidoptera: Crambidae) in the field (Phambala *et al.* 2020). Therefore, using pesticidal plants might promote ecosystem services and agro-ecologically sustainable crop production (Stevenson & Belmain 2017, Tembo *et al.* 2018).

Tephrosia vogelii is not only used as a traditional insecticide, but also serves as a fertilizer tree that improves soil fertility and health (Belmain *et al.* 2012, Grzywacz *et al.* 2014, Mkenda *et al.* 2014, Stevenson & Belmain 2017, Stevenson *et al.* 2012). Some reports suggest that it can also be used as an intercrop (Stevenson *et al.* 2012) to reduce FAW and other insect pest infestations through its repellent properties (Harrison *et al.* 2019, Kayange *et al.* 2019, Tembo *et al.* 2018). However, its efficacy needs to be substantiated by conducting more research (Harrison *et al.* 2019).

According to Mkenda *et al.* (2014), *T. vogelii* is highly effective in controlling numerous insect pests that are difficult to control such as cucumber beetle, leafhoppers, squash bugs, flea beetles, harlequin bug, spittle bugs, thrips, scales, mites, and some fruit worms. For effective control of pests of common bean, Mkenda *et al.* (2014) recommended that extracts of both neem tree and *T. vogelii* leaves be applied early in the morning or late in the afternoon to prevent biodegradation as a result of exposure to sunlight and air.

1.2.2 Using granular formulations of insecticides

1.2.2.1 Carbaryl GR

Application of carbaryl insecticide granules can provide effective control of FAW larvae and may be more effective than aqueous spray applications onto plant canopies. This is because granules are applied directly into the whorl where it remains in close proximity to where larvae feed deep inside the whorl (Pitre 1986). This also results in a reduced effect on natural enemies. Compared to aqueous sprays, the application of granular treatments is less harmful and less likely to be affected by precipitation after application (Pitre 1986). Varying levels of control of FAW by means of carbaryl insecticide granules have been reported in the USA as observed by Young (1979) and Pitre (1986). While Pitre (1986) reported low levels of control, Young (1979) reported both higher and low to zero levels of control on FAW larvae. In field trials with maize in Brazil, carbaryl was reported to be less effective against FAW (Pitre 1986). Sheets *et al.* (1982) also found that carbaryl was ineffective in controlling FAW on Coastal Bermuda grass in the south-eastern United States. This could partly be ascribed to Bermuda grass not having whorls in which larvae feed and in which carbaryl granules could remain for an extended period, within the feeding area of larvae.

1.2.2.2 Beta-cyfluthrin GR

Van den Berg and Van Rensburg (1993) evaluated the role of persistence and synergistic effects of various insecticides including beta-cyfluthrin GR for enhancing efficacy of control of *Chilo partellus* (Lepidoptera: Crambidae) on sorghum. Similar to FAW, *C. partellus* larvae also feed deep within maize whorls where they are not easily reached by insecticides (Slabbert & Van den Berg 2007). Their results showed that granular insecticide formulations were more effective than spray applications (Van den Berg & Van Rensburg 1993). Sylvian *et al.* (2015) evaluated the efficacy of whorl applications of granular insecticide formulations for control of maize stem borers (*B. fusca* and *C. partellus*) inside plant whorls where they exhibit similar feeding behaviour to that of FAW. In their study they applied ammonium nitrate (AN), Bulldock® 0.05GR (beta- cyfluthrin) and Dipterex® 2.5 GR (trichlofon). Bulldock® 0.05 GR effectively controlled *B. fusca* and *C. partellus* on maize and resulted in a reduction in leaf damage symptoms. The application of granular formulations of insecticides into maize and sorghum plant whorls therefore seem highly effective for control of pests that feed inside whorls, for example FAW.

1.2.3 Dust formulations of insecticides

1.2.3.1 Diatomaceous earth

Diatomaceous earth (DE) dust formulations are commonly used for protecting stored-products against insect pests (Arnaud 2003). The most extensive studies on the efficacy of DE have been conducted in the field of stored grain products (Shah & Khan 2014). Few studies have been conducted on its effects on FAW. Rios-Velasco *et al.* (2012) investigated the effects of DE formulations of the nucleopolyhedrovirus (NPV) on FAW and reported that application of a combination of NPV and DE resulted in higher levels of larval mortality compared to application of the virus only. Bohinc *et al.* (2018) investigated the efficacy of a DE formulation, SilicoSec®, against maize weevil and reported that surface treatment resulted in 100% adult mortality after seven days. Total mortality was also observed when maize weevils were exposed to wheat grain treated with SilicoSec®.

Arnaud (2003) examined the efficacy of four DE formulations, i.e., Insecto®, Perma-Guard™, Protect-It® and Dryacide® against red flour beetles and showed great variation in the efficacy of these products. Ziaee *et al.* (2016) investigated the insecticidal activity of four Iranian DEs against the saw toothed grain beetle, *O*ryzaephilus *surinamensis* (Coleoptera: Silvanidae) on wheat, barley and rice and recorded 100% mortality on wheat after a 10-day exposure period. Ziaee *et al.* (2016) observed that adult mortality increased with increased exposure time and concentration rate. Quarles (1992) reported that DE reduced the populations of European corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae), by 50% in field experiments. However, the yield of maize was also reduced due to delayed silking as a result of DE applications.

1.2.4 The use of soap-solutions

According to Babendreier *et al.* (2020), soap has been successfully used for controlling numerous insect pests, especially soft-bodied insects such as spider mites, thrips, mealybugs, whiteflies, psyllids and aphids. Although, the mode of action of soap-solutions is not well understood, it is known that it has contact effects on insects. Tembo *et al.* (2018) reported that soap has well-recognized effects on the cuticle and water regulation of arthropods. According to reports from farmers in Africa, soap-solutions applied into maize whorls can control FAW (Babendreier *et al.* 2020). Chhetri *et al.* (2019) found that soap solutions can also be used to destroy egg masses of FAW. However, this needs to be substantiated with research studies. Held *et al.* (2008) reported that a solution of dishwashing liquid resulted in immobilization of the FAW larvae while Tembo *et al.* (2018) also observed that a 0.1% soap solution applied into maize whorls significantly reduced larval numbers and yield loss due to FAW damage. However, the results of a study by Babendreier *et al.* (2020) reported that a local soap (African Black Soap, Alata Samina) was not effective against FAW since its application into plant whorls did not result in a reduction in larval survival and feeding damage. Forchibe *et al.* (2017) tested the same soap treatment at 2.5% w/v and reported it to be partially effective against aphids on cabbage in Ghana.

1.2.5 The use of soil/sand

Smallholder farmers apply soil/sand into whorls of maize plants to control FAW and report this to be effective (Yigezu & Wakgari 2020). The efficacy of sand and soil applications against FAW larvae inside plant whorls have been ascribed to both physical effects on larvae and a barrier effect which limits their movement. Desiccation of young larvae was the killing mechanisms described by Chhetri *et al.* (2019), while Babendreier *et al.* (2020) reported that soil might prevent insect pests from accessing the plant whorl and kill larvae through abrasion. This approach of applying sand/soil directly into leaf tissue whorls is reported to reduce yield losses incurred because of FAW infestation (Yigezu & Wakgari 2020) and has long been practiced by smallholder farmers in the Americas (Babendreier *et al.* 2020). Mixing dry sand with trichlorfon (powder or granules) and applying it into leaf whorls is also an effective approach that is practiced to control FAW in Ethiopia (Assefa & Ayalew 2019). Some farmers use ash in combination with conventional insecticides (Yigezu & Wakgari 2020) or apply sand before applying pesticides (Balabantaray and Samal 2021).

Although these methods are widely practiced by smallholder farmers who, based on field observations, consider it to provide some level of control, more research is needed to determine the efficacy and mechanisms of control of FAW and other insect pests (FAO 2018, Kansiime *et al.* 2019). A study conducted by Babendreier *et al.* (2020) in Ghana reported that the number of FAW larvae present in maize whorls treated with soil and ash, was low. However, it was not evident that plant damage could be mitigated, or if yield losses decreased after soil and ash treatments. Babendreier *et al.* (2020) concluded that applying ash, sand or soil into maize whorls did not show convincing efficacy against FAW.

1.2.6 The use of wood ash

Smallholder farmers in Africa use wood ash as a traditional method for controlling FAW (Assefa & Ayalew 2019). Applying wood ash into leaf whorls also results in the desiccation of young larvae (Chhetri *et al.* 2019) and reduces yield losses (Yigezu & Wakgari 2020). Babendreier *et al.* (2020) suggested that wood ash blocks the spiracles of FAW larvae, resulting in suffocation and death. Perera *et al.* (2019) also reported that application of wood ash into the whorls of maize plants was effective in reducing FAW numbers, but that there were practical difficulties in using such alternative methods at large scale.

Bohinc *et al.* (2018) investigated the insecticidal and contact effects of wood ash from three different tree species (Norway spruce, common beech and black locust) against adults of maize weevil under laboratory conditions and showed that all three treatments had significant contact effects on maize weevils. Wood ash can therefore be effectively used to protect stored-products from maize weevils. The efficacy of wood ash is accredited to many ingredients including SiO^2 which might enhance its hygroscopic properties. More research should be conducted to investigate the insecticidal properties of wood ash from various woody plant species to determine the most effective plant species (Bohinc *et al.* 2018).

1.3 Aim and objectives

Aim:

The aim of this study was to determine the efficacy of some of the alternative methods used by smallholder farmers to control *S. frugiperda* larvae.

Specific objectives were to:

- determine the efficacy of wood ash and soil, applied directly into whorls of maize plants, for control of *S. frugiperda* larvae,
- determine if wood ash and soil have repellent properties against *S. frugiperda* larvae under laboratory conditions (no-choice and two-choice tests),
- determine the effect of dry and wet wood ash on the migratory behaviour of *S. frugiperda* larvae on plants under laboratory conditions.

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Chapter 2

Evaluation of ash and soil for control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) on maize

Abstract

The sustainable cultivation of maize in Africa is threatened by the invasive fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) which was first reported in Africa in 2016. While insecticides can be used for control of this pest, chemical control is difficult due to its cryptic feeding behaviour. Insecticide application, especially if done by smallholder farmers with little experience and poor access to appropriate insecticides, often do not provide desirable results and is a threat to human health. The application of soil and ash as alternative control methods have been suggested but its efficacy has not been scientifically evaluated. The aim of this study was to determine the efficacy of ash and soil applied directly into maize whorls for control of FAW. Results showed that application of soil and ash provided sufficient control of larvae. Whorl application of ash resulted in significant mortality of 1st and 5th-instar larvae inside plant whorls under laboratory conditions. Mortality of 5th-instar larvae that was treated with dry ash was 62.5%, while that of the control treatment was 20.83%. High reduction in survival of 3rd-instar larvae inside plant whorls was obtained five days after the application of different treatments under field conditions. Application of dry ash, soil and wet ash resulted in larval survival of 21.4, 25.0 and 33.9%, respectively, while larval survival on untreated plants was 69.6%. Application of dry and wet ash, 24 hrs after inoculation of neonate larvae into maize whorls resulted in 67.5 and 65.6% mortality after 5 days, respectively, compared to the control (22%). Preventative application of dry ash before inoculation of neonate larvae into maize whorls resulted in 68.9% mortality, while that of the control was 37.6% after five days. There was a significant reduction in larval feeding damage over the 5-day period after application of soil, wet and dry ash. Soil, wet and dry ash exhibited non-preference effects against FAW larvae in no-choice and two-choice bioassays. Whorl application of soil, wet and dry ash were found to be effective against FAW larvae and holds potential for use by smallholder farmers as alternatives to chemical control.

Key words: maize pests, alternative control methods, integrated pest management, smallholder farmers

2.1 Introduction

Fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) which was first reported in Africa in 2016, threatens the production of maize by smallholder farmers in Africa (Goergen *et al.* 2016). The most effective and commonly used control method for this pest is the application of synthetic insecticides (Rios-Velasco *et al.* 2012, Kumela *et al.* 2018, Tambo *et al.* 2020). The level of control achieved by insecticide application is often reported to be poor (Al-Sarar *et al.* 2006, Makale *et al.* 2022, Suguiyama *et al.* 2020).

The management of FAW is difficult due to its cryptic feeding behaviour and wide host range, rapid reproduction (Assefa & Ayalew 2019) and multiple generations per year (Yigezu & Wakgari 2020). The efficacy of chemical control is influenced by larval behaviour since they feed deep inside maize whorls, making it difficult to reach the target by means of spray applications (Carvalho *et al.* 2013, Young 1979). Furthermore, insecticides are not effective when not applied during the susceptible stages of the insect's life cycle, which, in this case implies the first 5 – 7 days after egg hatch (Yu *et al.* 1983). Effective chemical control of FAW largely depends on farmers' knowledge of the pest, insecticides and application methods. Unfortunately, smallholder farmers have little experience in the handling and application of insecticides (Bateman *et al.* 2018, Jepson *et al.* 2020). Synthetic insecticides also pose adverse impacts on human health and the environment (Jepson *et al.* 2020, Yigezu and Wakgari 2020). Furthermore, access to personal protective equipment for use when handling pesticides is very limited, and farmers also use a wide range of redundant or banned insecticides and have limited access to modern and safer pesticides (Jepson *et al.* 2020).

Therefore, it is necessary to promote alternative pest control methods such as pesticidal plants or application of wood ash and soil because these are applicable to smallholder farmers as they carry low-risks and are affordable (Belmain *et al.* 2012, Grzywacz *et al.* 2014, Maredia *et al.* 1992). Smallholder farmers do apply soil/sand into whorls of maize plants and report this to be effective (Yigezu & Wakgari 2020). Applying sand/soil directly into plant whorls is reported to reduce yield losses incurred as a result of FAW infestation (Yigezu & Wakgari 2020) and has long been practiced by smallholder farmers in the Americas (Babendreier *et al.* 2020). The efficacy of wood ash applied for control of FAW was reported in Sri Lanka by Perera *et al.* (2019), who also highlighted that this method could be applicable in smallholder farming systems. However, there is still a need for conducting more research to determine the efficacy of these alternative control methods against FAW larvae (FAO 2018, Kansiime *et al.* 2019).

The aim of this study was to determine the efficacy of wood ash and soil applied directly into the whorls of maize plants for control of FAW larvae, and the effects of these materials on the behaviour of larvae. The optimum time of application of wood ash and soil, i.e., prior to infestation with neonate larvae (preventative application) and after larval infestation (curative application) was also determined.

2.2 Material and methods

Rearing of fall armyworm larvae

A population of FAW was collected by pulling out the whorls of infested plants in maize fields at Groblersdal (Limpopo Province) (S 25° 16′ 28.21″ E 29° 25′ 23.74″) in February 2021. The collected larvae were then maintained at the Entomology Laboratory of North-West University, where they were reared on artificial diet and maize whorl leaf tissue until they pupated. Pupae were collected and individually transferred into 50 ml containers covered with mesh lids to allow for air flow until the moths emerged. Male and female moths were then paired for mating and put into plastic oviposition containers (38 cm × 27 cm × 14.5 cm) covered with mesh lids. Wax paper was put inside the oviposition containers as an oviposition substrate. A 10% sugar solution was provided in cotton buds as a source of energy for moths. Egg batches were collected on daily basis and transferred into 50 ml containers until they hatched. After the eggs hatched, the neonate larvae were transferred into 500 ml plastic rearing containers containing artificial diet or maize whorl tissue leaves. Containers were covered with mesh lids and larvae were reared under constant climate conditions at a temperature of 28 ºC, RH of 70% and a photoperiod of 14L: 10D. This rearing procedure was followed throughout the course of this study.

Study site

All the experiments were conducted at the Entomology Laboratory of the North-West University, Potchefstroom campus, South Africa. Semi-field experiments were conducted in a shade house and plant growth tunnel in which seed was planted directly into the soil. Field experiments were conducted in an experimental field.

General experimental design

Three pilot trials were conducted after which eight laboratory assays and three field experiments were conducted.

All experiments commenced when plants were in the mid-whorl stage, four weeks after seedling emergence. Plants were artificially inoculated with FAW larvae in all experiments using a fine camel hairbrush.

The soil material for application into maize whorls was collected from a maize field at the university. Wood ash was obtained by burning firewood of different tree species. These included Eucalyptus sp. and Acacia sp. Ash was then sieved using a 2 mm aperture sieve to separate the finer particles from other material. Wet wood ash was prepared by mixing wood ash with water.

2.2.1 Pilot experiments

General experimental design

Three semi-field pilot experiments were conducted to determine appropriate amount (dosage) of ash and soil to apply per plant whorl, as well as the volume of water to use in experiments where wet wood ash were to be used as treatments. These pilot experiments were conducted in a plant growth tunnel and shade house using potted plants. Maize variety, PHB 30Y83 was used for all pilot experiments.

2.2.1.1 The volume of water required for preparation of wet wood ash

The aim of this pilot experiment was to determine the volume of water that could be applied into maize whorls without killing a significant number of larvae in treatments where wet ash was used. This was done in order to prevent obtaining inaccurate results of larval mortality due to drowning and not as a result of the wet ash treatment.

The experimental design was a randomized complete block. Maize was planted in 10 litre plastic pots. Each pot contained five plants. Each plant was artificially inoculated with twenty 1st-instar larvae. Larvae were allowed 24 hours to establish after which treatments were applied by pouring water directly into the whorls. Larval feeding damage was checked prior to the application of treatments to ensure the presence of larvae. Treatments comprised of different volumes of water, i.e., 0 ml (control), 20 ml, 40 ml, 60 ml and 100 ml per maize whorl. Each treatment was replicated two times, and each replicate comprised of ten plants. Larval survival was determined 48 hours after treatment by cutting and dissecting plants and recording the number of live larvae per plant.

2.2.1.2 The dosage rate of wood ash required for control of 1st-instar FAW larvae

The aim of this experiment was to determine the required dosage rate of ash for effective control of FAW larvae. The experiment was conducted in a shade house using a randomized complete block design. Maize was planted in 20 litre pots with four plants in each pot. The experiment had four treatments (dosage rates) of wood ash. These were 0 g (control), 5 g, 10 g and 15 g per maize whorl. Each treatment was replicated four times and each replicate consisted of 20 plants. Each plant was artificially inoculated with twenty 1st-instar larvae. Larvae were placed directly inside the whorl and wood ash was also applied directly into the whorl, 24 hours after inoculation. Plants were checked for feeding damage which indicated the presence of the larvae before applying treatments. Plants were dissected five days after ash was applied and the number of surviving larvae per plant was recorded.

The treatment that resulted in the highest mortality was determined and that volume (g) of ash was then used in all further experiments described in this dissertation.

2.2.1.3 The dosage rate of soil required for control of 1st-instar FAW larvae

This experiment was designed to determine the amount of soil required for effectively controlling FAW larvae in plant whorls. The layout of the experiment was a randomized complete block design. There were four treatments (dosage rates) of soil, i.e., 0 g, 5 g, 10 g, and 30 g of soil per maize whorl. Each treatment had four replicates and each replicate consisted of eleven plants. The experiment was conducted using maize plants in twenty litre pots. Each pot comprised of four plants.

Each plant was inoculated with twenty 1st-instar larvae which were placed inside the whorls, followed by application of soil after 24 hours. Plants were examined for feeding damage before treatments were applied to ensure that the inoculated larvae established in plant whorls.

Larval survival was recorded five days after application of soil by dissecting plants and recording the number of live larvae per plant. The treatment that resulted in the highest mortality was determined and that volume (g) of soil was then used in field experiments described in this dissertation.

2.2.2 Laboratory assays: The influence of whorl application of wood ash on the mortality and ballooning behaviour of FAW larvae

General experimental design

Maize cultivar CRN3505 was used in all assays in which the effect of application of ash into plant whorls on larval behaviour and mortality were assessed. The experimental design for all experiments was a randomized complete block design. Two maize seeds were planted per 5 litre plastic pot which were maintained under greenhouse conditions. Each experiment commenced four weeks after seedling emergence and were conducted under laboratory conditions.

The incidence of ballooning behaviour of larvae was recorded by placing card board underneath plants to trap larvae that left the plants. Card board sheets (70 cm × 50 cm) were covered with yellow sticky trap sheets and served as a catchment surface area beneath the plants to trap ballooning larvae (Fig. 2.1). An incision was made at the center of the cardboard to fit around the stems of plants. Leaves that were hanging outside the catchment surface area were trimmed to ensure that all larvae were trapped within the catchment surface area to prevent larvae from falling onto the ground and escaping unnoticed.

Figure 2.1: Sticky cardboard beneath maize plants for trapping larvae that ballooned off the plants.

The experiments comprised of three treatments, i.e., control, dry ash and wet ash. The treatment of wet ash used throughout the experiments consisted of 200 g of ash in 1000 ml of water. The volume of water that was used for mixing with wood ash was obtained from the water volume pilot experiment. Wet ash was applied as a full whorl-coverage. In the case of dry ash treatments, 10 g per plant whorl was applied, as indicated by the results of the pilot experiments. All plants were observed to detect ballooning larvae (migratory behaviour). Observations were made at 0 min, 30 min, 1.5 hr, 2.5 hr and 4.5 hr after inoculation/treatment. Larvae that ballooned off from plants were trapped on the sticky cardboard placed beneath plants. The number of larvae that ballooned onto sticky traps were recorded by making a mark on the sticky surface where the larva was trapped. The percentage larval mortality due to the ash treatment inside plant whorls was calculated after subtracting the number of larvae that ballooned off from plants, from the number that was inoculated onto plants.

Temperature data was collected using iButton temperature data loggers.

2.2.2.1 The effect of preventative application of wood ash on FAW larval mortality and ballooning behaviour in 1st-instars

The experiment had three treatments (control, wet ash and dry ash). Each treatment comprised of twelve plants, and each plant served as a replicate, resulting in twelve replicates per treatment. Treatments were applied by pouring either dry or wet ash into the whorls of maize plants prior to inoculation with larvae. Each plant was then inoculated by placing fifty 1st-instar larvae on the leaves above the ash, immediately after treatment. Plants were observed at 0 min, 30 min, 1.5 hr, 2.5 hr and 4.5 hr after inoculation/treatment to record the number of ballooning larvae. Larvae that ballooned off from plants onto the sticky cardboards were recorded at each time interval until the experiment was terminated 48 hrs after inoculation. Notes on the movement behaviour of the larvae were also taken during observations. The number of surviving larvae per plant was recorded by dissecting plants and larval mortality was calculated as described above.

2.2.2.2 The effect of curative application of wood ash on FAW larval mortality and ballooning behaviour in 1st-instars

The treatments of used in this experiment were a control, wet ash and dry ash. Each treatment comprised of twelve replicates with one plant per replicate. Plants were artificially inoculated with fifty 1st-instar larvae each. Larvae were given a period of 24 hours to establish before the plants were treated. Treatments were then applied 24 hours post artificial inoculation. Feeding damage was checked on each plant before applying treatments to ensure that larvae established in the plant whorls. Observations on larval ballooning behaviour were made and the number of larvae that ballooned off from plants was recorded at similar time intervals as in the previous experiment. Notes on the movement behaviour of larvae were taken at each time interval until the experiment was terminated 48 hours after treatment. Plants were dissected and number of surviving larvae was recorded as described above.

2.2.2.3 The effect of curative application of wood ash on FAW larval mortality and escape behaviour in 3rd-instars

The experiment comprised of three treatments which were a control, wet ash and dry ash. Each treatment had twelve replicates of one plant each. Plants were artificially inoculated with 3rd-instar larvae. Larval instar was determined based on larval age (7 days) and development at 28 ºC, RH of 70% and a photoperiod of 14L: 10D (Schlemmer 2018). Each plant was inoculated with only four 3rd-instar larvae to minimise the chances of cannibalism. Treatments were applied 24 hours after artificial inoculation. Plants were inspected for the presence of larvae before applying the treatments. Observations on migratory behaviour and the number of larvae that escaped from plants onto sticky cardboards were recorded over two days (48 hours). The number of surviving larvae per plant was recorded after four days (96 hours).

2.2.2.4 The effect of curative application of wood ash on larval mortality and escape behaviour in 5thinstars

The treatments of this experiment were control, wet ash and dry ash. Twelve plants were used in each treatment and each plant served as a replicate, resulting in a total of twelve replicates per treatment. Larval instar was determined based on larval age (14 days) and development at constant climatic conditions (28 ºC, RH of 70% and a photoperiod of 14L: 10D) (Schlemmer 2018). Each plant was artificially inoculated with two 5th-instar larvae to reduce cannibalism. Ash treatments were applied 24 hours after inoculation with 5th-instar larvae to give larvae sufficient time to establish inside plant whorls. Plants were checked for feeding damage prior to the application of treatments. Migratory and larval escape behaviour was observed for a period of 48 hours after treatment. Plants were cut and dissected, and the number of surviving larvae per plant recorded.

2.2.3 Laboratory bioassays: FAW larval preference for maize leaves treated with wood ash and soil

General experimental design

The aim of this experiment was to assess larval preference and repellent effects of ash and soil treatments. Laboratory bioassays were conducted in 90 cm petri dishes using no-choice and two-choice tests. The experiment was conducted under constant climate conditions at a temperature of 28 ºC, RH of 70% and a photoperiod of 14L: 10D.

2.2.3.1 No-choice tests with 1st-instar larvae

The experiment comprised of four treatments, i.e. control, wet ash, dry ash and soil. Each treatment was replicated four times with twenty petri dishes per replicate. Square leaf cuttings (discs) of 3 cm × 3 cm were cut from maize furl leaf tissue. Treatments were then applied onto maize leaf discs. Maize leaf discs were treated by rubbing them gently with either dry ash or soil on both sides. The wet ash treatment was applied by dipping the leaf discs into the wet ash solution after which they were allowed to dry before they were used. Both ash and soil were sieved prior to treating the leaf discs.

Each maize leaf disc was attached to the centre of a 7 cm × 6 cm piece of paper using paper clips (Fig. 2.2). This was done to hold the leaf discs in place and to prevent them from moving and folding over. Each maize leaf disc was then placed at the centre of a petri dish. Five neonate larvae were put on top of each leaf disc using a fine camel hairbrush. Petri dishes were then sealed with parafilm to prevent larvae from escaping.

Larval preference for different treatments were determined based on how larvae responded to the treatments after a certain period of exposure. The positions of larvae inside petri dishes were recorded after 1, 4 and 24 hours to determine their responses as described by Leiner and Spafford (2016). Larvae that were not present on or underneath the leaf disc, were recorded as no-choice reactions and were excluded from the data analysis (Leiner & Spafford 2016).

Figure 2.2: The set-up of the no-choice tests.

2.2.3.2 No-choice tests with 3rd-instar larvae

This experiment followed similar methods as described above. The treatments were a control, wet ash, dry ash and soil. Each treatment had four replicates comprising of twenty petri dishes each. Treated leaf discs were attached onto a piece of paper as described above and placed at the centre of the petri dishes. One 3rd-instar larva was then placed on top of each leaf disc. Parafilm was used to seal the petri dishes. Experiments were conducted under constant climatic conditions as described above. Observations were made after 1, 4 and 24 hours and the position of larvae recorded. Larvae that were not present on the leaf disc were recorded as no-choice reactions and were not included in the data analysis.

2.2.3.3 Two-choice tests with 1st-instar larvae

The following combinations of treatments were evaluated in the 2-choice tests: control vs dry ash, control vs wet ash, and control vs soil. Experiments were replicated four times and each replicate comprised of 20 petri dishes.

Four leaf discs (1.5 x 1.5 cm) were attached with paper clips onto a square of a 7 cm × 6 cm piece of paper (Fig. 2.3). Two leaf discs were treated with one of the treatments while the other two representing the control were not treated. Leaf discs of a similar treatment were placed opposite each other at a distance of 1.5 cm from each other. Leaf discs were put into petri dishes and five neonate larvae were placed at the centre of the petri dish and allowed to choose between the two treatment combinations. Petri dishes were sealed with parafilm.

Observations were made and the positions of larvae were recorded at 1, 4 and 24 hours after inoculation. Larvae that did not choose any of the two treatment combinations were recorded as no-choice and were disregarded from data analysis.

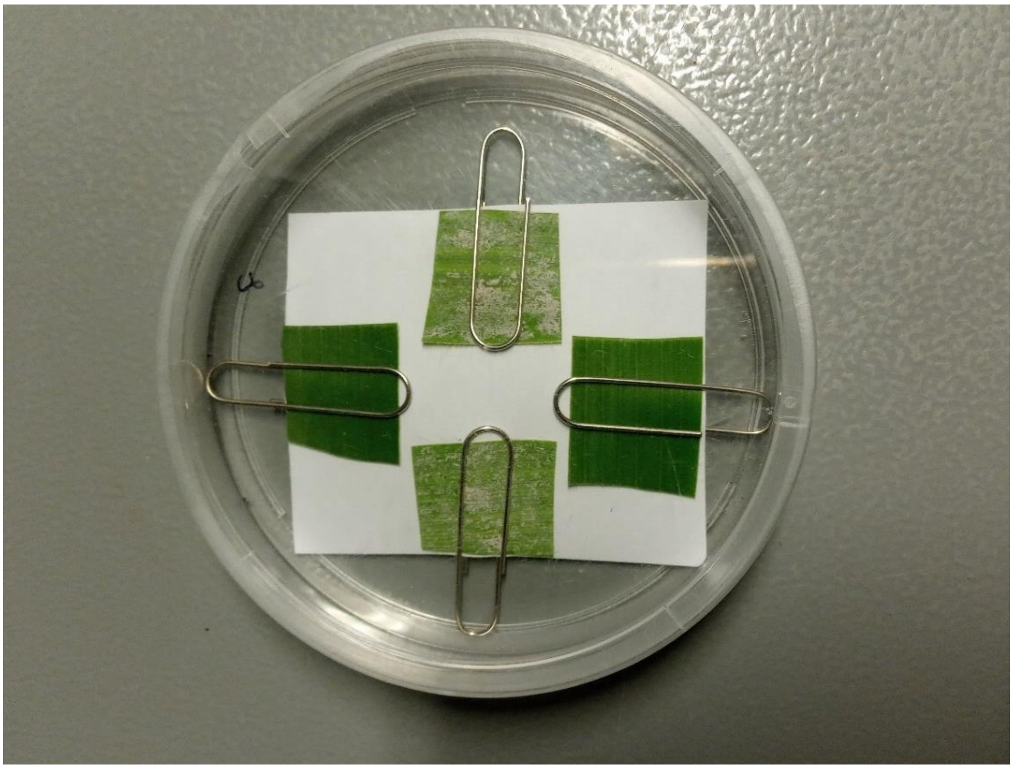

Figure 2.3: The set-up of the two-choice tests.

2.2.3.4 Two-choice tests with 3rd-instar larvae

The following combinations of treatments were evaluated in the 2-choice tests: control vs dry ash, control vs wet ash, and control vs soil. Treatments were replicated four times and each replicate comprised of 20 petri dishes.

Four leaf discs of two different treatments were attached onto the sides of a piece of paper and put into petri dishes. One 3rd-instar larva was then placed at the centre of the petri dish and left to choose between the two treatment combinations. Experiments were observed at similar time intervals as mentioned above and the position of the larvae was recorded. Larvae that made no choice between the two treatment combinations were counted as no-choice and were not considered in data analysis. Climatic conditions were kept constant and similar to that of the experiments described above.

2.2.4 Field experiments: the efficacy of whorl application of wood ash and soil for control of FAW larvae on maize.

General experimental design

Three field trials were conducted. The experimental design was a randomized complete block. The soil type of the experimental field was red loam soil, and the field was ploughed and seed planted by hand. Weeds were removed by hand throughout the experiment period. Plants were watered as necessary.

2.2.4.1 Efficacy of wood ash and soil for control of 3rd-instar larvae

Maize was planted during October 2021 using an open pollinated variety (Kalahari). The inoculation with larvae was done in November 2021. The field trial comprised of five rows of maize plus two side rows that were used as border rows. Each row was 15 m long, with an inter-row spacing of 90 cm and intra-row spacing of 10 cm. The first and last plant of each row served as border plants and were not inoculated. Treatments within a row were separated by two plants. Data loggers were used to record temperature and relative humidity.

The field trial commenced four weeks after seedling emergence and comprised of four treatments. These were: control, wet ash, dry ash and soil. Each treatment was replicated five times with seven plants per replicate. Each plant was artificially inoculated with two 3rd-instar larvae which were placed inside the whorl using a fine camel hairbrush. Larvae were given a period of 24 hours to establish in plant whorls before treatments were applied. Therefore, plants were treated 24 hours after inoculation by pouring the treatments directly into the whorl. The dosage rates of treatments used in this experiment were obtained through pilot experiments that were conducted to determine the most effective dosage rate. For the dry ash treatment, 10 g was applied per whorl and for the soil treatment, 30 g was applied per whorl. The wet ash treatment was applied as full whorl-coverage.

Larval survival was recorded after five days by dissecting the plants and determining the number of live larvae per plant.

2.2.4.2 Efficacy of dry wood ash and soil for control of 1st-instar larvae

The aim of this field trial was to determine the efficacy of both curative and preventative control applications of ash and soil against 1^{st}-instar larvae of FAW.

Planting took place in February 2022 and inoculation with larvae was done in March 2022 when plants were four weeks old. Maize variety CRN3505 was used in this experiment. The field trial had five rows that were 15 m in length with a spacing of 90 cm between rows and an inter-row spacing of 10 cm.

The trial had six treatments of which three were applied as a preventative control measure (prior to infestation) and the other three as a curative control measure (post infestation). The treatments were control, dry wood ash and soil. Each treatment comprised of five replicates and each replicate had seven plants. For curative control, each plant was artificially inoculated with twenty 1^{st}-instar larvae per whorl followed by application of treatments into whorls 24 hours after inoculation, using similar application rates as described above. For preventative control, treatments were applied into the whorl prior to artificial inoculation. Plants were then immediately inoculated by placing larvae on the leaves not inside the whorl as opposed to the curative control. Each plant was inoculated with twenty 1^{st}-instar larvae. The trial was terminated after five days when the number of surviving larvae and feeding damage was recorded. Larval feeding damage to the furl leaves was rated using the Davis scale (0- 9 rating scale) (Table 2.1) (Davis *et al.* 1992).

2.2.4.3 Efficacy of wet and dry wood ash for control of 1st-instar larvae

The aim of this experiment was to determine the optimal application time as well as to compare the effects of wet and dry ash for control of 1^{st}-instar larvae. Therefore, a field trial was conducted.

This experiment followed a similar methodology as the previous experiment. Maize variety, CRN3505 was planted in October 2021, and the plants were inoculated with larvae in November 2021. The field trial consisted of five rows which were 15 m long with an inter-row and intra-row spacing of 90 cm and 10 cm, respectively. The trial had six treatments i.e., control, wet ash and dry ash and each treatment was applied both as curative and preventative control. There were five replicates per treatment and each replicate consisted of seven plants. Treatments were applied exactly as described above. For preventative control treatments, applications were made into the whorls, after which each plant was artificially inoculated with twenty 1st-instar larvae. However, for curative control, each plant was inoculated with twenty 1st-instar larvae inside the whorl, and then treatments were applied inside the whorl 24 hours after plants were inoculated. The field trial was terminated after five days when feeding damage was scored and the number of live larvae per plant recorded. The Davis scale (0- 9 rating scale) was used to score larval feeding damage on the mid-whorl tissue leaves (Table 2.1) (Davis *et al* 1992)

Table 2.1: Description of the rating scale for fall armyworm larval feeding damage on maize mid-whorl leaf tissue based on visual estimation according to the 0 - 9 Davis scale (Davis *et al.* 1992).

Score	Description
0	No feeding damage visible
1	Only pinhole lesions present on whorl leaves
2	Pinhole and small circular lesions present on whorl leaves
3	Pinholes, small circular lesions and a few small elongated lesions that are rectangular shaped of up to 1.3 cm in length present on the whorl and furl leaves
4	Small elongated lesions present on the whorl leaves and a few medium-sized elongated lesions of 1.3 to 2.5 cm in length present on the whorl and furl leaves
5	Small elongated lesions and numerous medium-sized elongated lesions present on the whorl and furl leaves
6	Small and medium-sized elongated lesions and a fewer huge elongated lesions that are bigger than 2.5 cm in length present on the whorl and furl leaves
7	Several small and medium-sized elongated lesions present on the whorl and numerous big elongated lesions present on the furl leaves
8	Many small and medium-sized elongated lesions present on the whorl and many huge elongated lesions present on the furl leaves
9	Several elongated lesions of different sizes on whorl and furl leaves and a few uniform to irregular shaped holes eaten from the base of the whorl and/or furl leaves.

2.3 Statistical analysis

Data on larval survival in the preliminary experiments with volume of water and dosage rates for soil and ash, were tested for normality by means of the Shapiro-Wilk test and for homogeneity of variance, using Levene's test. Data on the volume of water and soil dosage rates met these assumptions and were subsequently analysed using one-way analysis of variance (ANOVA). Comparisons amongst different treatments were done using Tukey HSD test. Larval survival data in the preliminary experiment testing the ash dosage rates, were neither normally distributed nor homogeneous. This data was therefore analysed by means of the Kruskal-Wallis test.

The percentage larval mortality in behavioral studies was calculated as follows: the number of larvae that ballooned off from plants was subtracted from the initial number of larvae inoculated onto plants to obtain the actual number of larvae that remained per plant. The number of live larvae per plant was then divided by the actual number of larvae that remained per plant (did not balloon off) and multiplied by 100 to calculate percentage survival. One-way ANOVA was conducted thereafter.

Data on the incidence of larval ballooning were subjected to one-way ANOVA. Data on larval ballooning behaviour in the experiment conducted to determine the efficacy of curative applications against 1st-instar larvae were neither normally distributed nor homogenous. Therefore, it was analysed using the Kruskal-Wallis test.

Larval response data in no-choice tests at 1, 4 and 24 hours were expressed as proportions and data for each test were analysed by means of binomial distribution tests, followed by Bonferroni correction to adjust for multiple-mean comparisons. The proportion of larvae exhibiting different preferences between and within treatments was also compared over time. Feeding preference responses in the two-choice tests were also analysed by means of binomial distribution tests, and choices made were tested against a 50 % preference ratio.

Larval survival data for all three field experiments were analysed by means of one-way ANOVAs, following the same analysis used for the preliminary experiments. The mean damage rating score per plot was calculated for each treatment and data subjected to one-way ANOVAs.

All analyses were conducted using TIBCO Software® 14.0.0.15 (TIBCO Software Inc., 2020).

2.4 Results

2.4.1 Preliminary experiments

2.4.1.1 The volume of water required for preparation of wet ash

The mean percentage of surviving larvae was not significantly different in all five treatments (Table 2.2). The 60 ml treatment had the lowest survival (48.00% ± 3.15), while the highest survival rate was obtained on the 100 ml treatment which had a mean percentage of 61.75 ± 4.30. Overall, the results of this experiment indicated that the different water volumes that were tested had no influence on the survival of 1st-instar FAW larvae. It was therefore decided to use the dosage of 200 g (ash)/1000 ml (water) for the ash solution (wet ash treatment) in this study.

Table 2.2: Mean number of surviving 1st-instar FAW larvae inside maize whorls 48 hours after the application of different volumes of water.

Treatment volume (ml/plant)	Mean larval survival (%) ± SE
0	60.50 ± 4.75
20	59.50 ± 5.10
40	60.00 ± 4.45
60	48.00 ± 3.15
100	61.75 ± 4.30

Means were not significantly different ($p>0.05$)

2.4.1.2 The dosage rate of ash required for control of 1st-instar FAW larvae

The results of the multiple comparisons of p-values by means of the Kruskal Wallis test showed no significant differences amongst the four treatments ($H(3) = 3.59$ and $P = 0.31$). Since there were no differences in the percentage mortality caused by the different dosages of ash treatments (0, 5, 10 and 15 g), it was decided to use 10 g of dry ash in all the experiments that were conducted thereafter.

2.4.1.3 The dosage rate of soil required for control of 1st-instar FAW larvae

The mean percentage of larvae that survived after 5 days in the 5 and 30 g treatments were 11.25% ± 2.79 and 11.93% ± 4.67, respectively. The levels of survival did, however, not differ significantly between treatments (Table 2.3). Since there were no differences between treatments, it was decided to use a dose of 30 g of soil per plant in all field experiments that were conducted thereafter.

Table 2.3: Mean number of surviving 1st-instar FAW larvae per maize whorl, five days after treatment with different dosage rates of soil.

Treatment mass (g/plant)	Mean larval survival (%) ± SE
0	22.27 ± 1.83
5	11.25 ± 2.79
10	20.68 ± 5.15
30	11.93 ± 4.67

Means were not significantly different ($p>0.05$)

2.4.2 Laboratory assays: The influence of whorl application of wood ash on mortality and ballooning/escape behaviour of FAW larvae

2.4.2.1 The effect of preventative application of ash on FAW larval mortality and ballooning behaviour in 1st-instars

Larval mortality

Significant differences ($p = 0.003$) were observed in the mortality of larvae between the different preventative treatments (Table 2.4). Larval mortality in the dry ash treatment did not differ significantly from the wet ash treatment, however, it differed significantly from the untreated control. The mean larval mortality in the dry ash treatment was 68.96%, while wet ash resulted in larval mortality of 53.55% (Table 2.4).

Table 2.4: Mean percentage mortality of 1st-instar FAW larvae inside maize whorls, 48 hours after the application of wet and dry ash prior to artificial inoculation of larvae.

Treatment	Mean larval mortality (%) ± SE
Control	37.64 ± 4.39 a
Wet ash	53.55 ± 7.68 ab
Dry ash	68.96 ± 5.00 b

Means within a column followed by a different letter differs significantly ($p<0.05$).

Larval migratory (ballooning) behaviour

The number of larvae that ballooned off from whorl leaves did not differ significantly between treatments ($p = 0.75$). Although the dry ash treatment had the higher mean percentage (21.17%) of larvae that ballooned off, the differences were not significant from the control and wet ash treatments (Table 2.5).

Table 2.5: Mean number of 1st-instar FAW larvae ballooning off from maize whorls treated with wet and dry ash over a period of 48 hours after inoculation.

Treatment	Mean incidence of larval ballooning (%) ± SE
Control	18.00 ± 2.38
Wet ash	19.67 ± 2.72
Dry ash	21.17 ± 3.60

Means were not significantly different ($p>0.05$)

2.4.2.2 The effect of curative application of ash on FAW larval mortality and ballooning behaviour in 1st-instars

Larval mortality

There was a significant difference ($p = 0.00001$) between the treatments that were tested for efficacy of curative control of 1st-instar larvae (Table 2.6). The dry and wet ash treatments resulted in similar levels of mortality, which was also significantly higher than that observed in the control (22.08%). The mean larval mortality for dry and wet ash was 67.45% and 65.64%, respectively (Table 2.6).

Table 2.6: Mean percentage mortality of 1st-instar FAW larvae, 48 hours after application of wet and dry ash into maize whorls.

Treatment	**Mean larval mortality (%) ± SE**
Control	22.08 ± 4.50 a
Wet ash	65.64 ± 3.46 b
Dry ash	67.45 ± 4.04 b

Means within a column followed by a different letter differs significantly (p<0.05).

Larval migratory (ballooning) behaviour

The number of 1st-instar FAW larvae that ballooned off from maize whorls treated with wet and dry ash applied after artificial inoculation differed significantly between treatments (H (2) = 8.96 and P = 0.01) (Table 2.7). The numbers of larvae that ballooned off from plants receiving dry ash treatment were significantly lower than that in the control and wet ash treatment. However, the numbers of larvae that ballooned off from plants that received wet ash treatment did not differ significantly from the control treatment.

Table 2.7: Mean number of 1st-instar FAW larvae ballooning off from maize whorls treated with wet and dry ash over a period of 72 hours after inoculation.

Larvae that ballooned off from the plants	Multiple Comparisons p values (2 tailed) Kruskal-Wallis test: H (2, N= 34) = 8.96 P= 0.0113 Treatment		
	Control R: 20.58	Wet ash R: 20.96	Dry ash R: 9.65
Control		1.00 a	0.03 b
Wet ash	1.00 a		0.02 b
Dry ash	0.03 b	0.02 b	

2.4.2.3 The effect of curative application of ash on FAW mortality and escape behaviour in 3rd-instars

Larval mortality

The efficacy of curative control applications against 3rd-instar larvae did not differ significantly ($p = 0.43$) between treatments (Table 2.8). Wet and dry ash resulted in low mean larval mortality, which did not differ significantly from that of the control.

Table 2.8: Mean percentage mortality of 3rd-instar FAW larvae, 96 hours after application of wet and dry ash into maize whorls.

Treatment	Mean larval mortality (%) ± SE
Control	23.61 ± 6.14
Wet ash	27.78 ± 7.20
Dry ash	36.81 ± 8.30

Means were not significantly different ($p>0.05$)

Larval migratory (escape) behaviour

The number of 3rd-instar larvae that escaped from the plants did not differ significantly between treatments ($p = 0.66$) (Table 2.9). The incidence of larvae that escaped from plants ranged from 10.5 to 14.5% for the ash treatments, compared to the 18.75% of the control treatment.

Table 2.9: Mean number of 3rd-instar FAW larvae that escaped from maize plants over a five-day period, after application of wet and dry ash.

Treatment	Mean incidence of larval escape (%) ± SE
Control	18.75 ± 7.00
Wet ash	14.50 ± 7.25
Dry ash	10.50 ± 4.75

Means were not significantly different ($p>0.05$)

2.4.2.4 The effect of curative application of ash on FAW larval mortality and escape behaviour in 5th-instars

Larval mortality

The level of larval mortality differed significantly ($p = 0.009$) between the treatments. Dry ash provided the highest mortality of 5th-instar FAW larvae (62.25%) (Table 2.10). Wet ash did not achieve effective control of 5th-instar larvae and the levels of mortality in the control and wet ash treatments did not differ significantly.

Table 2.10: Mean percentage mortality of 5th-instar FAW larvae, 48 hours after application of wet and dry ash into maize whorls.

Treatment	Mean larval mortality (%)
Control	20.83 ± 7.43 a
Wet ash	27.27 ± 10.37 a
Dry ash	62.50 ± 10.88 b

Means within a column followed by different letters differs significantly ($p<0.05$).

Larval migratory (escape) behaviour

There were no significant differences between the incidences of escape of 5th-instar larvae from maize whorls treated with wet and dry ash (Table 2.11). There was however a tendency that more larvae escaped from plants that received the dry ash treatment, although the differences were not significant.

Table 2.11: Mean incidence of 5th-instar FAW larvae that escaped from maize whorls treated with wet and dry ash over a period of 72 hours after artificial inoculation.

Treatment	Mean incidence of larval escape (%) ± SE
Control	8.50 ± 5.50
Wet ash	12.50 ± 9.00
Dry ash	21.00 ± 7.50

Means were not significantly different ($p>0.05$)

2.4.3 Laboratory bioassays: FAW larval preference for maize leaves treated with ash and soil

2.4.3.1 No-choice tests with 1st-instar FAW larvae

A larger proportion of larvae preferred to settle on leaf tissue of the control and soil treatments, compared to the ash treatments 1 hr after larvae were exposed to the different treatments (Fig. 2.4). However, after 4 and 24 hours, higher numbers of larvae preferred to settle on leaf tissue treated with soil. These differences were, however, not significant (Table 2.12). There was a general tendency that leaves treated with ash were less preferred compared to the control and soil treatments (Fig. 2.4). Both the control and soil treatments were significantly more preferred at 1, 4 and 24 hours (Fig. 2.4 and Table 2.12), whereas dry and wet ash was the least preferred throughout the entire duration of the experiment (Fig. 2.4). There was a significant difference in the numbers of larvae that settled on leaf tissue treated with dry and wet ash after 1 and 4 hours but not at 24 hours (Table 2.12).

Larval preference within each treatment were also compared over time. The proportions of larvae that settled on leaf tissue of the different treatments at 1, 4 and 24 hours are provided in Table 2.13. The number of larvae that responded positively towards leaves treated with dry ash decreased significantly over time (Fig. 2.4 and Table 2.13). This was contrary to the wet ash and soil treatments, where the number of positively responding larvae increased significantly over time (Fig. 2.4 and Table 2.13).

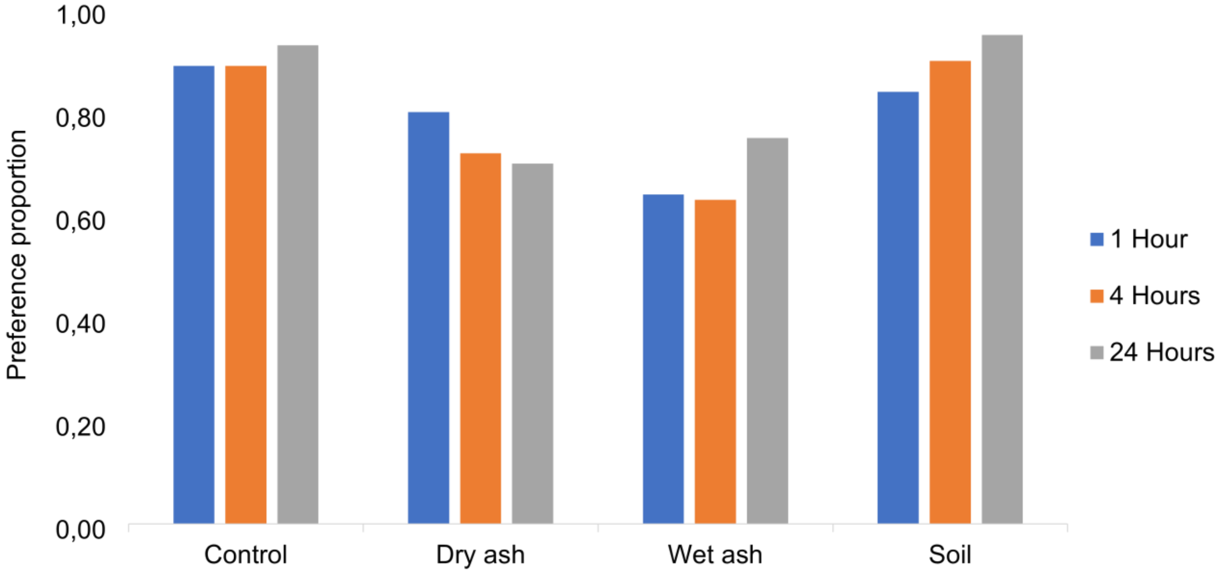

Figure 2.4: The response of 1st-instar FAW larvae in no-choice tests, 1, 4 and 24 hours after treatment with dry ash, wet ash and soil.

Table 2.12: Results of binomial distribution test of 1st-instar FAW larval responses after 1, 4 and 24 hours in no-choice tests (Bonferroni correction was used).

Treatment	**P-value**		
	1 hour	**4 hours**	**24 hours**
Control vs. dry ash	0.002	0.000	0.000
Control vs. wet ash	0.000	0.000	0.000
Control vs. soil	0.300	3.870	1.400
Dry ash vs. wet ash	0.000	0.04	0.680
Dry ash vs. soil	0.850	0.000	0.000
Wet ash vs. soil	0.000	0.000	0.000

P-values in red indicate significant difference

Table 2.13: Results of binomial distribution test of 1st-instar FAW larval responses after 1, 4 and 24 hours in no-choice tests (Bonferroni correction was used). Data overtime were compared per treatment.

Treatment	Evaluation times (in hours)	Comparison of choice at different evaluation times	P-Value
Control	1 x 4 x 24	1 X 4	NS
		1 X 24	NS
		4 X 24	NS
Dry ash	1 x 4 x 24	1 X 4	*
		1 X 24	**
		4 X 24	NS
Wet ash	1 x 4 x 24	1 X 4	NS
		1 X 24	**
		4 X 24	***
Soil	1 x 4 x 24	1 X 4	*
		1 X 24	***
		4 X 24	*

Significance indicated by *P<0.05; **P<0.01; ***P<0.001 and NS = no significant difference.

2.4.3.2 No-choice tests with 3rd-instar larvae

Larvae exhibited the highest preference for untreated leaves after 1 and 4 hours, whereas dry ash was the second most preferred treatment (Fig. 2.5). However, there were no significant differences between the two treatments after the different time periods (Table 2.14). Wet ash-treated maize leaves were least preferred, followed by leaves treated with soil (Fig. 2.5). Consequently, wet ash and soil were the least preferred with no significant differences between these two treatments at 1 and 4 hours (Table 2.14). Leaf tissue of the wet ash treatment had the highest larval preference at 24 hours, followed by soil and dry ash (Fig. 2.5). However, no significant differences were observed between the wet ash, dry ash and soil treatments at 24 hours (Table 2.14). The control treatment had the lowest numbers of larvae at 24 hours and significant differences were observed between the control and wet ash treatments. However, there were no significant differences between the control, soil and dry ash at 24 hours.

Larval preferences at different time intervals within treatments were compared over time (Table 2.15). The number of larvae that responded positively towards the untreated leaves decreased over the 24 hr period. However, the differences were not significant.

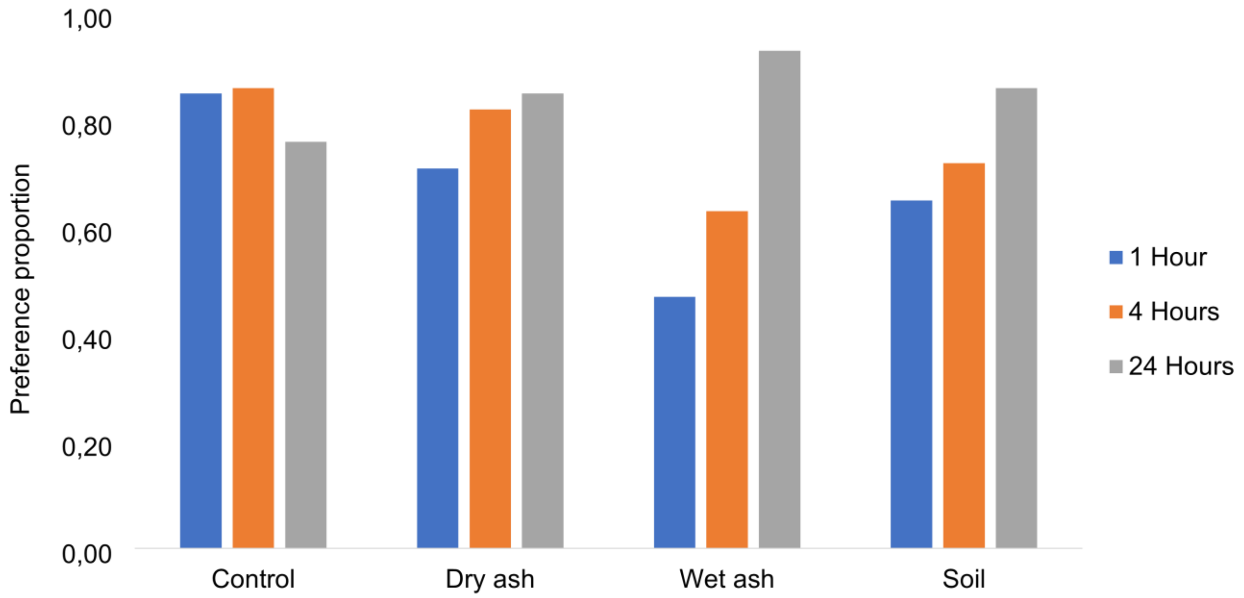

Figure 2.5: The response of 3rd-instar FAW larvae, at 1, 4 and 24 hours in no-choice tests with maize leaf tissue treated with dry ash, wet ash and soil.

Table 2.14: Results of binomial distribution test of data on 3rd-instar FAW larval responses in no-choice tests after 1, 4 and 24 hours.

Treatments	**P-value**		
	1 hour	**4 hours**	**24 hours**
Control vs. dry ash	0.190	2.940	0.900
Control vs. wet ash	0.000	0.005	0.02
Control vs. soil	0.02	0.180	0.640
Dry ash vs. wet ash	0.01	0.04	0.640
Dry ash vs. soil	2.490	0.790	5.140
Wet ash vs. soil	0.130	1.350	0.890

P-values in red indicate significant difference

Table 2.15: Results of binomial distribution test of data on 3rd-instar FAW larval responses in no-choice tests after 1, 4 and 24 hours. Data overtime were compared per treatment.

Treatment	Evaluation times (in hours)	Comparison of choice at different evaluation times	P-Value
Control	1 x 4 x 24	1 X 4	NS
		1 X 24	NS
		4 X 24	NS
Dry ash	1 x 4 x 24	1 X 4	NS
		1 X 24	NS
		4 X 24	NS
Wet ash	1 x 4 x 24	1 X 4	NS
		1 X 24	***
		4 X 24	***
Soil	1 x 4 x 24	1 X 4	NS
		1 X 24	**
		4 X 24	NS

Significance indicated by *P<0.05; **P<0.01; ***P<0.001 and NS = no significant difference.

2.4.3.3 Two-choice tests with 1st-instar larvae

The preference of larvae for untreated maize leaf tissue was significantly higher than that for dry ash at 1, 4 and 24 hours (Figures 2.6). Similarly, leaves treated with wet ash and soil were significantly less preferred than those of the untreated maize leaf tissue at 1, 4 and 24 hours. Larval preference for the untreated maize leaf tissue increased over time, while that of the dry ash and soil decreased. The number of larvae which did not make a choice between untreated leaves and leaves treated with dry ash was significantly higher after 1 hour (Fig. 2.6 a), in contrast to 4 hours (Fig. 2.6 b) and 24 hours (Fig. 2.6 c). The number of larvae which made no choice between the untreated and soil-treated leaves decreased over the 24-hour period. Significantly high numbers of larvae settled on the untreated leaf material in all the choice-combinations, at all three time intervals.

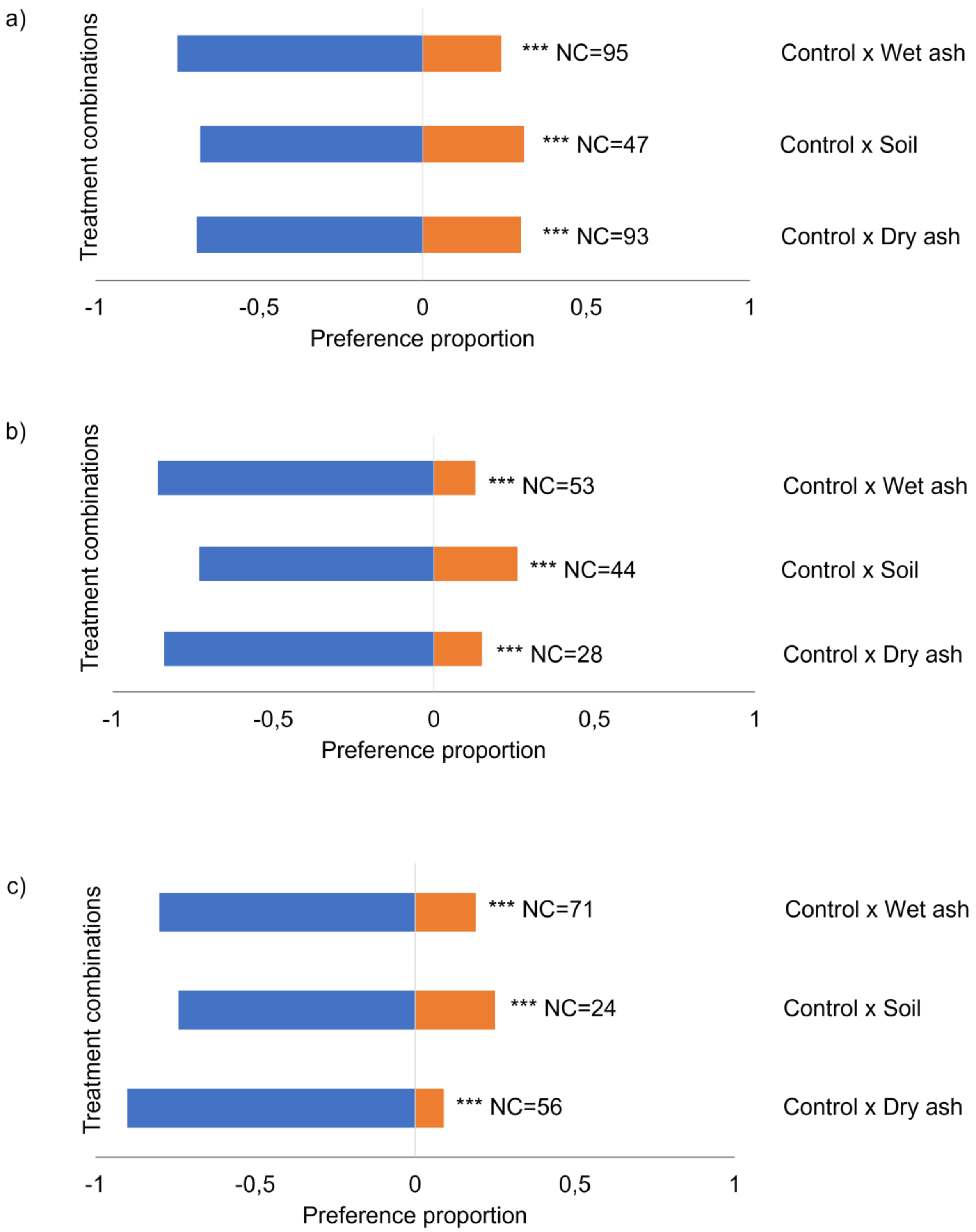

Figure 2.6: The proportions of 1st-instar FAW larvae settling on maize leaf tissue treated with dry ash, wet ash and soil in two-choice tests after, a) 1 hr, b) 4 hr, and c) 24 hr. Significance indicated by *p<0.05; **p<0.01 and ***p<0.001. 'NC' indicates the number of larvae (out of 400 per combination) that did not make a choice.

2.4.3.4 Two-choice tests with 3rd-instar larvae

The untreated maize leaf tissue was significantly preferred to maize leaf tissue treated with dry ash, wet ash and soil at 1, 4 and 24 hours (Figures 2.7). Consequently, the untreated maize leaf tissue had high larval preferences in all the two-choice combinations at all time intervals (1, 4 and 24 hours). Larval preferences for untreated maize leaf tissue increased greatly over time in all the combinations, whereas only a slight increase over time was observed for maize leaf tissue treated with wet ash and soil. However, the proportion of larvae that settled on maize leaf tissue treated with dry ash remained similar over time. The number of larvae which did not make any choice in the two-choice assays with untreated and dry ash-treated leaf tissue was greater than the number of larvae that made a choice after 1 and 4 hours. In addition, the proportion of larvae that did not make a choice was very high in all the two-choice combinations after 1 and 4 hours. However, large numbers of larvae settled on untreated maize leaf tissue after 24 hr.

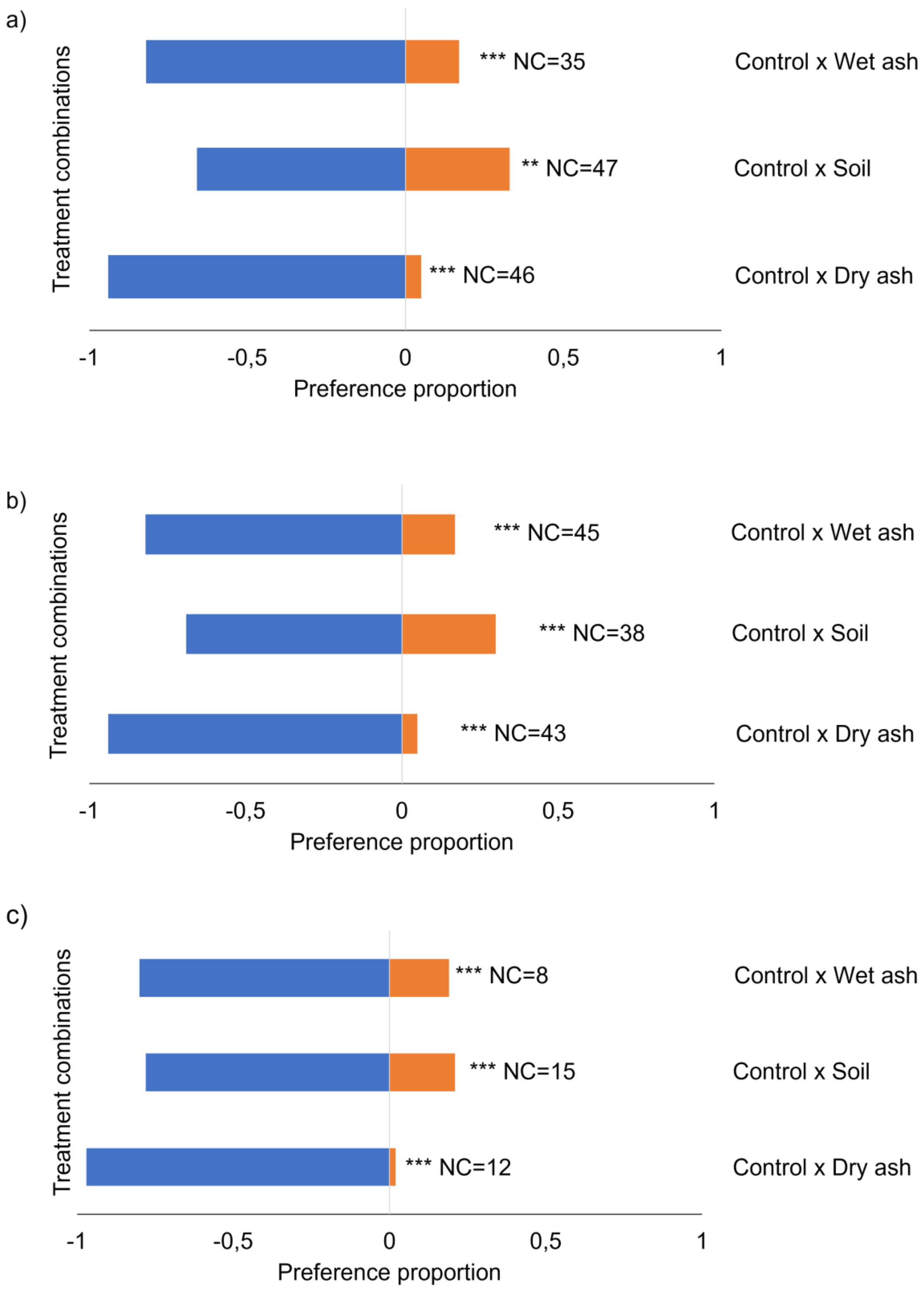

Figure 2.7: The proportions of 3rd-instar FAW larvae settling on maize leaf tissue treated with dry ash, wet ash and soil in two-choice tests after, a) 1 hr, b) 4 hr and c) 24 hr. Significance indicated by *p<0.05; **p<0.01 and ***p<0.001. 'NC' indicates the number of larvae (out of 80 per combination) that did not make a choice.

2.4.4 Field experiments: the efficacy of whorl application of ash and soil for control of FAW larvae on maize.

2.4.4.1 Efficacy of ash and soil for control of 3^{rd}-instar larvae

The percentage survival of 3^{rd}-instar larvae differed significantly between the treatments (p = 0.00066) (Table 2.16). All the treatments resulted in significant reductions in the numbers of surviving larvae compared to the control. The percentage of surviving larvae after five days ranged from 21.4 to 33.9% and did not differ significantly between the dry ash, wet ash and soil treatments.

Table 2.16: Mean percentage survival of 3^{rd}-instar larvae, five days after curative treatment with soil, wet ash and dry ash.

Treatment	Mean larval survival (%) ± SE
Control	69.64 ±6.07 a
Wet ash	33.93 ± 6.79 b
Soil	25.00 ± 7.43 b
Dry ash	21.43 ± 5.07 b

Means within a column followed by a different letter differs significantly (p<0.05).

2.4.4.2 Efficacy of dry ash and soil for control of 1st-instar larvae

Larval survival

Mean larval survival in this experiment ranged from 18.57 to 38.00%. There were no significant differences (p = 0.12) between the different treatments that were tested for efficacy of curative or preventative control (Table 2.17). Although the untreated control treatment in the curative applications had the higher number of surviving larvae and soil that was applied as a curative control measure had the lowest number of surviving larvae, the differences were however not significant between treatments.

Table 2.17: Mean percentage survival of 1st-instar FAW larvae inside maize whorls, five days after application of soil and dry ash as preventative control measures, and five days after application into whorls as curative control measure.

Treatment	Mean larval survival (%) ± SE
Control-curative	38.00 ± 3.00
Dry ash-curative	28.29 ± 6.54
Soil- curative	18.57 ± 2.90
Control-preventative	26.00 ± 2.86
Dry ash-preventative	23.71 ± 4.84
Soil-preventative	27.29 ± 5.62

Means were not significantly different (p>0.05)

Larval feeding damage

Significant differences in larval feeding damage symptoms were observed between treatments (p= 0.000002). The lowest mean feeding damage rating of 3.26 was recorded in the treatment where soil was applied as a preventative treatment (Table 2.18). Preventative application of soil was more successful, followed by curative and preventative applications of dry ash. However, the differences between the three treatments, i.e., soil (preventative) and dry ash (both curative and preventative) were not significant. Curative application of soil did not result in significant differences in larval feeding damage between the control treatments (curative and preventative) (Table 2.18). Moreover, significant differences were observed between curative and preventative application of soil.

Table 2.18: Mean rating score of FAW damage to maize whorl leaves. Damage was scored five days after application of soil and dry ash in the preventative and curative control.

Treatment	Mean damage rating score ± SE
Soil-preventative	3.26 ± 0.14 a
Dry ash-curative	3.34 ± 0.13 ab
Dry ash-preventative	3.37 ± 0.15 ab
Soil- curative	3.89 ± 0.14 bc
Control-curative	4.14 ± 0.15 c
Control-preventative	4.14 ± 0.19 c

Means within a column followed by a different letter differs significantly ($p<0.05$)

2.4.4.3 Efficacy of wet and dry ash for control of 1st-instar larvae

Larval survival

No significant differences in the number of surviving larvae were observed between treatments ($p = 0.396$). The curative applications of wet and dry ash resulted in low larval survival in contrast to the preventative applications of wet and dry ash but the differences were not significant. The two untreated control treatments of the curative and preventative control applications had the high larval survival, although the differences were not significant from the other treatments (Table 2.19).

Table 2.19: Mean survival of 1st-instar FAW larvae inside maize whorls, five days after the application of wet and dry ash as preventative and curative control.

Treatment	Mean larval survival (%) ± SE
Control-curative	12.71 ± 2.74
Wet ash- curative	6.86 ± 2.17
Dry ash-curative	5.57 ± 1.73
Control-preventative	10.86 ± 2.45
Wet ash-preventative	8.14 ± 2.58
Dry ash-preventative	7.29 ± 3.51

Means were not significantly different ($p>0.05$)

Larval feeding damage

Larval feeding damage in the different treatments differed significantly ($p = 0.001$) (Table 2.20) and both dry and wet ash had a strong influence on larval feeding damage. This was indicated by a significant reduction in feeding damage on plants that were treated with wet and dry ash. This was similar to the results of the previous experiment with soil and dry ash. Feeding damage rating of plants treated with dry ash (preventative application), wet ash (curative application) and wet ash (preventative application) were 2.62, 2.71 and 2.71, respectively (Table 2.20). Preventative application of dry ash resulted in the lowest degree of damage.

Table 2.20: Mean rating score of FAW damage to maize whorl leaves. Damage was scored five days after application of soil and dry ash in the preventative and curative control treatments.

Treatment	Mean feeding damage rating score ± SE
Dry ash-preventative	2.62 ± 0.16 a
Wet ash- curative	2.71 ± 0.12 ab
Wet ash-preventative	2.71 ± 0.16 ab
Dry ash-curative	3.38 ± 0.19 bc
Control-preventative	3.52 ± 0.20 cd
Control-curative	4.14 ± 0.23 d

Means within a column followed by the same letter do not differ significantly ($p>0.05$).

2.5 Discussion

The laboratory results of the current study showed that whorl application of dry ash (10 g/plant) has potential to effectively control 1st-instar FAW larvae. High levels of larval mortality were recorded in both curative (after inoculation) and preventative (before inoculation) applications of dry ash. Curative application of wet ash (full whorl-coverage) also resulted in high mortality of 1st-instar FAW larvae. Therefore, based on the laboratory results of the present study, application of ash before and after infestation can successfully control 1st-instar larvae. This would consequently reduce feeding damage and possibly yield loss by limiting the survival of large numbers of larvae into late instars (3^{rd} to 6^{th} instars) that are more damaging. These results indicated that ash has a potential to provide partial solutions to smallholder farmers who suffer yield losses as a result of FAW infestation in their crops. However, the effects of ash on yield loss still needs to be evaluated through studies assessing efficacy of control on maize ears.

Although the laboratory experiments of the present study successfully reduced larval survival of 1^{st}-instar FAW larvae, field experiments for both preventative and curative whorl applications of soil as well as dry and wet ash were however unsuccessful in reducing 1^{st}-instar FAW larval survival. These results were not in correspondence with the results of the laboratory experiments in which wet and dry ash provided effective control of 1^{st}-instar FAW larvae. Although whorl applications of soil, wet and dry ash did not reduce survival of 1^{st}-instar larvae under field conditions, larval feeding damage was significantly reduced in both curative and preventative whorl applications. This indicated that whorl applications of these materials limits FAW feeding and damage to leaves. This was also observed in larval preference tests where ash and soil showed strong non-preference effects against 1^{st} and 3^{rd}-instar larvae in two-choice tests.

It was observed in the field experiments that significantly large numbers of 1^{st}-instar larvae that were artificially inoculated into plant whorls disappeared from plants in all treatments. This made it impossible to obtain accurate results on larval survival because all treatments, including the untreated control, had similarly low levels of larval survival in terms of the numbers inoculated onto plants. The experiments were therefore repeated twice and were conducted in two different growing seasons, i.e., September to November 2021, and January to March 2022. However, accurate results could not be obtained in both experiments due to very low larval survival that was obtained from all treatments since most of the larvae disappeared. The two seasons in which the field experiments were conducted had remarkable heavy strong winds. First-instar FAW larvae get dispersed by wind through ballooning using silk threads (Suby *et al.* 2020). Therefore, this scenario could be an indication of what happens under natural conditions and could possibly be ascribed to the behaviour of 1^{st}-instar FAW larvae.

Furthermore, Zalucki *et al.* (2002) reported that first instars of most lepidopteran species disappear from plants soon after hatching, and in most cases, these are assumed to be dead. Mortality in neonate larvae of Lepidoptera is very high, though it differs greatly in different environments. Larvae that disappeared from the untreated control treatments in the November 2021 trials ranged between 80 - 90 %, while for March 2022, the range was between 60 - 90%. Zalucki *et al.* (2002) reported that the number of lepidopteran 1st-instar larvae that get lost in the field is between 9 and 96%. This explains why such a large number of 1st-instar larvae disappeared in the field experiments of the present study as opposed to laboratory experiments. Predation, disappearance, dispersal, and weather are some of the mortality factors that impact on numbers of first instars. However, in most cases the cause of disappearance and mortality remains unknown and is not always explainable, partly because 1st-instar larvae are very small (Zalucki *et al.* 2002).

Du Plessis *et al.* (2020) reported that the optimal temperature range for FAW in South Africa was between 26 and 30 °C, while the minimum temperature threshold for larval development was 12.12 °C. In the present study, the mean average temperature that was recorded during the field experiments with dry ash and soil was 25.36 °C with a RH of 40.66% for September to November 2021 (data not shown), and 21.70 °C with RH of 72.91% for January to March 2022. During the field experiments with wet and dry ash, the mean average temperature was 24.55 °C with a RH of 45.78% and 20.15 °C with RH of 74.36% for September to November 2021 and January to March 2022 (data is not shown), respectively. These temperatures were above the minimum temperature threshold (12.12 °C) for FAW larval development (Du Plessis *et al.* 2020) and indicated that temperature was not the causal factor for low larval survival or disappearance of larvae in the present study. Therefore, it was concluded that the disappearance of 1st-intsar FAW larvae in the field experiments of the present study was due to natural migration behaviour through ballooning, in response to various factors such as overcrowding. However, in the laboratory studies, ash treatments did not induce increased migration of larvae, possibly due to confinement as a result of ash, which limited the migration of larvae especially 1st-instar larvae as they were buried under ash and therefore were unable to migrate. In addition, migration of 1st-instar FAW larvae through ballooning is induced or aided by wind, and therefore, low levels of migration in the laboratory studies were also ascribed to a confined laboratory environment which prevented ballooning through wind as opposed to field experiments.

Dry ash that was applied as a curative control measure strongly reduced the migration of 1^{st}-instar FAW larvae in the laboratory studies of the present study. A significantly lower number of larvae ballooned off from plant whorls that were treated with dry ash, since neonate larvae were trapped inside the whorl. The low number of larvae that ballooned off from the whorl indicated that curative application of dry ash hindered larvae from ballooning. Both larvae (Suby *et al.* 2020) and adults of FAW migrates through wind dispersal (Day *et al.* 2017). Dispersal through ballooning or crawling enables larvae to move from one plant to another, which leads to redistribution of the insect population which may further increase the incidence of infested plants at the field level (Sokame *et al.* 2020). Therefore, curative application of dry ash could therefore be used to limit the dispersal of neonate larvae away from natal plants, provided that the application is done within a day or two after eggs have hatched. Preventative application of dry and wet ash did not have a significant influence on the migration behaviour of 1^{st}-instar FAW larvae compared to the control. However, preventative application of dry ash can prevent 1^{st}-instar FAW larvae from getting inside the whorl after hatching. The presence of a physical barrier of ash, combined with the non-preference effects of ash and soil observed in this study, may therefore, provide significant protection of maize plants against FAW feeding damage.

Visual observations of the migration behaviour of 1^{st}-instar larvae under laboratory conditions showed similar patterns in all treatments that were applied preventatively. Some larvae dispersed by crawling all over the leaf blades of the plant while some larvae were suspended from leaves by means of silk threads. These larvae eventually ballooned off the plants onto the sticky trap sheets that were placed underneath the plants. However, some larvae were observed climbing back onto plants using the silk thread. Sokame *et al.* (2020) reported similar movement behaviour and that larvae climb back onto plants by means of the silk threads. Little larval activity was observed 24 hours after treatment/inoculation, as most of the larvae settled on the leaf blades in all treatments. Some larvae moved to the insides of the whorls of plants that received the wet ash treatment and the untreated control since feeding damage was later observed on the whorl leaf tissue. Similar movement patterns of 1st-instar larvae, i.e., dispersal within the plants, hanging by silk threads and ballooning were observed on plants that received the curative and preventive control treatments. However, for curative control, some larvae were observed moving upwards within the whorl leaf tissue after the application of dry and wet ash. The movement patterns that were observed after the dry ash treatment indicated that some larvae escaped the dry ash since they were not covered when dry ash was applied. Therefore, they were able to freely move around since it is impossible for 1st-instar larvae to move through the ash and come out of the whorl and thereafter balloon off the plants, which was the case with 3rd and 5th-instar larvae. Some of the larvae were found moving upwards on the outer whorl leaves, while some were moving on the stems of the plants in the control and wet ash treatments.

Both wet and dry ash did not have a significant impact on the migration behaviour of 3rd and 5th-instar FAW larvae. Less larval movement (activity) were observed on plants treated with dry ash, in contrast to wet ash and the untreated control. It was evident that both wet and dry ash repelled FAW larvae since larvae were observed climbing upwards on the whorl leaves after the application of wet ash, but remained within the whorl on the upper part where there was no contact with wet ash. Some larvae climbed off the whorl and settled outside the whorl on the leaf blades in the wet ash treatment. In the case of the dry ash treatment, larvae were observed crawling through the dry ash and emerging amongst the whorl leaves after which they would come out of the whorl (escape) and fall onto the sticky trap. After crawling through dry ash, some of the larvae would move upwards towards the edge of the whorl leaf where there was no dry ash. This behaviour was common in both 3rd and 5th-instar larvae. Some of the larvae were also observed coming out of the whorl through feeding holes that they bored into the tightly folded whorl leaves on plants treated with dry ash. Overall, ballooning and escape was observed to be common in all larval stages including 3rd and 5th-instar larvae. These larval movement patterns indicated that wet and dry ash can disturb larvae by repelling them off the whorl. Although large larvae (3rd to 6th-instars) usually dwell inside the whorl where they hide and feed (Suby *et al.* 2020), they were observed coming out of the whorl and settling on the leaf blades after application of wet and dry ash. This could expose larvae to natural enemies, thereby facilitating biological control.

Preferences of 1st-instar FAW larvae in no-choice tests indicated that dry ash possessed strong repellent effects as opposed to other treatments. This tendency was confirmed by a significant reduction in the number of larvae that responded positively towards dry ash over time. Two-choice tests with 1st-instar larvae also indicated that soil, wet and dry ash could repel FAW larvae and reduce feeding damage. This was indicated by a large proportion of 1st-instar larvae that showed high preference towards the control, while soil, wet and dry ash were least preferred. This was also observed for 3rd-instar larvae in two-choice tests. Findings of the preference tests of the present study are in line with the findings of Hailu *et al.* (2021) who reported that maize leaf tissue treated with ash did not have any feeding damage symptoms under laboratory conditions in Malawi. This indicated that ash carries repellent effects towards FAW larvae by hindering them from feeding on leaves treated with ash.

Results of the field experiments on whorl applications of soil and ash as a curative control measure against 3rd-instar FAW larvae showed that all the treatments were effective in reducing larval survival in contrast to laboratory experiments. These results showed that these alternative control methods employed by smallholder farmers against FAW larvae can be effective as indicated by a significant reduction in survival of 3rd-instar FAW larvae under field conditions after the application of soil (30 g/whorl), dry ash (10 g/whorl) and wet ash (full whorl-coverage). Dry ash was the most effective treatment in reducing larval survival. This was followed by soil treatment and wet ash against 3rd-instar FAW larvae under field conditions. Dry ash also resulted in high mortality of 5th-instar FAW larvae under laboratory conditions, in contrast to wet ash. However, in this study, experiments on 5th-instar larvae were not continued under field conditions because 5th-instar larvae were observed climbing off the whorl and crawling through the ash of plants used in the laboratory experiments.

The results of the present study were in accordance with results of a study in India which reported that whorl applications of soil, sand and ash, at 5 g per plant, provided effective control of FAW larvae (Varma *et al.* 2021). Varma *et al.* (2021) also reported that these treatments reduced the incidence of FAW larvae and provided a yield gain and increased dry fodder yield. Furthermore, whorl applications of soil and sand could also provide protection of maize ears as Varma *et al.* (2020) indicated that it significantly reduced ear damage caused by FAW in India. Similar results were reported by Lyle *et al.* (2021) in the Limpopo Province of South Africa. The latter authors showed that applications of ash effectively reduced ear damage on maize plants which received an application of ash into the whorls at weekly intervals from the V1-stage onwards (Lyle *et al.*, 2021).

This, as well as other studies have shown that these alternative control methods can protect maize in many ways by repelling FAW larvae and reducing the incidence of larvae which leads to reduction in feeding damage and consequently, reduction in yield loss. Varma *et al.* (2020) reported that soil and sand were more effective than ash, whereas in the current study, dry ash was the most effective treatment in reducing larval survival. Kamnyamata (2020) reported that ash resulted in 80% reduction in feeding damage by FAW larvae under field conditions. The latter study reported that reduced feeding damage resulted in a yield of 2500 kg/ha on plots that were treated with ash, in contrast to untreated plots that yielded 1000 kg/ha (Kamnyamata 2021). The results of the present study also showed a significant reduction in feeding damage by 1st-instar FAW larvae under field conditions. Donga *et al.* (2021) also indicated that soil, sand, ash and fish soup is highly effective in controlling FAW larvae. The efficacy of soil applications have been found to be comparable to that of some synthetic insecticides, e.g. chlorpyrifos, by obtaining similar grain yield (Donga *et al.* 2021). The efficacy of ash have been also documented for other insect pest species such as the maize weevil (Bohinc *et al.* 2018). Bohinc *et al.* (2020) reported that ash showed contact and insecticidal effects on the maize weevil. This indicated that ash can be effectively used both in the field and in stored-products.

The female moth of FAW lays eggs in masses of up to 200 eggs per batch with a total of up to 2000 eggs per female (Balabantaray and Samal 2021). After egg hatch, larvae disperse on the natal plant and to neighbouring plants for feeding. Initial feeding by large numbers of 1st-instar larvae may cause significant leaf injury (Britz 2020). However, feeding damage by 1st-instar FAW larvae is usually not clearly visible because of the minute size of the larvae, and therefore, it is restricted to pinholes and small lesions that does not inflict yield loss (Britz, 2020). Consequently, this type of feeding damage is often not detected in time and control is either not applied or applied at a too late stage (too large larvae). This then provides 1st-instar larvae the opportunity to develop and cause severe feeding damage (Britz 2020) because the degree of feeding and damage increases as larvae become bigger (Suby *et al*. 2020). Control measures such as the application of chemicals at late larval stages is usually ineffective (Britz 2020). Therefore, application of control measures against early larval stages (1st-instar) of FAW is essential in order to kill late-instars that inflict severe damage which subsequently results in huge yield losses. Furthermore, controlling small larvae (1st to 3rd-instars) is relatively easy compared to large larvae (4th to 6th-instars) (van den Berg *et al.* 2021). Applications of soil, as well as wet or dry ash into maize whorls may therefore efficaciously reduce both the survival and feeding damage caused by early-instar larvae as shown in this study.

According to van den Berg *et al.* (2021), the most susceptible stages of maize plants to FAW feeding damage are during seedling emergences and the early whorl stages. Therefore, during the early whorl stages, plants require intensive protection to reduce yield loss. Van den Berg *et al.* (2021) reported that protecting maize plants against FAW larval feeding damage during early vegetative growth stages results in a huge yield gain. Soil and ash carries no potential harm towards the environment (Varma *et al.* 2021) and proved to be effective when applied as preventative control measures against 1st-instar larvae in the present study. Therefore, it can be applied as a preventative control measure during seedling emergence and early whorl stages to kill FAW larvae and protect maize from severe feeding damage. The implementation of preventative measures has been reported to be the best and most efficacious approach towards controlling FAW larvae (Balabantaray and Samal 2021). Balabantaray and Samal, (2021) also reported that whorl applications of soil, ash and sand + lime should be initiated during seedling to early whorl stages when 5% of the plants show damage symptoms. Reduction in foliar damage has been also reported through whorl application of ash (Khangati *et al.*, 2021). In addition, the level of infestation can have a strong influence on yield loss if no control measures are applied (van den Berg *et al.*, 2021).

Some studies reported that these alternative control methods have the potential to enhance the efficacy of insecticides when used in conjunction with insecticides. This further indicates the significance of these alternative methods since some smallholder farmers in Africa still apply insecticides against FAW larvae as indicated in farmer’s surveys (Bateman *et al.* 2021, Kansiime *et al.* 2019, Kumela *et al.* 2019, Tambo *et al.* 2019, Tepa-Yotto *et al.* 2022). Divya *et al.* (2021) reported that sand can be used synergistically with insecticides when it is applied as a ‘’granular’’ product.

Sand mixed with insecticides can reduce the amount of insecticides required per unit area by 50%, in contrast to foliar insecticide spray applications. Reduction in feeding damage and higher grain yields can also be obtained when insecticides are mixed with sand and used for control of FAW larvae (Divya *et al.* 2021). Furthermore, sand increases the efficacy of insecticides by enhancing its longevity inside maize whorls and exerting additional physical effects on the cuticle of larvae since it is in direct contact with larvae inside the whorl (Divya *et al.* 2021). The use of chemicals in conjunction with ash has also been reported to be more effective than using chemicals alone (Kassie *et al.* 2020). Balabantaray and Samal (2021) also reported that applying soil, ash and sand mixed with lime before applying insecticides can enhance the efficacy of insecticides on larger FAW larvae that dwells deep inside the whorl. The efficacy of ash was found to be similar to that of *T. vogelii* (a pesticidal plant also used against FAW) (Kamnyamata 2021).

The present study and other studies have shown that whorl applications of ash, soil and sand can provide effective control of FAW larvae (Varma *et al.* 2021). Therefore, recommendations have been made that these control methods can be incorporated as elements of Integrated Pest Management (IPM) programs for managing FAW by smallholder farmers (Varma *et al.* 2021). These control methods are not costly and are considered environmentally friendly and can therefore protect the environment (Varma *et al.* 2021) from chemical residues that runoff to the environment as a result of misuse. Preservation of natural enemies that suppress pest numbers can also be achieved with the use of these alternative control methods (Shinde *et al.* 2021). For example, Shinde *et al.* (2021) reported that whorl applications of ash and sand + lime was safe for natural enemies such as predatory bugs, earwigs and lady beetles, in contrast to the application of insecticides such as carbofuran granules. Application of ash and soil could also minimize the use of insecticides by smallholder farmers since it carries health risks due to unavailability of appropriate safety precaution measures (Day *et al.* 2017, Sisay *et al.* 2019a). In addition, the negative impacts of insecticides on natural enemies could be minimized (Divya *et al.* 2021) through the adoption of these local innovations as alternative control methods. However, the efficacy of these alternative control methods such as whorl applications of sand, soil and ash to control FAW larvae on maize can provide variable results as observed in the laboratory and field experiments of the present study.

The mechanism of control provided by the application of sand + lime and soil is described as the abrasion of the soft cuticle of FAW larvae, which eventually leads to death as a result of desiccation (Balabantaray and Samal 2021, Divya *et al.* 2021). Ash kills insects by suffocating them as it blocks their spiracles (Babendreier *et al.* 2020). Sand and soil minimizes the effects of FAW larvae on maize by entrapping the larvae (Divya *et al.* 2021) and inhibiting their access into plant whorls (Babendreier *et al.* 2020, Divya *et al.* 2021). Soil, ash and sand does not affect plant growth as it slowly comes out of the plant as the plant matures (Divya *et al.* 2021).

2. 6 Conclusion

Soil and ash have the potential to provide control of FAW at different larval stages (1st and 3rd-instar). The efficacy of these alternative control methods was observed to differ between laboratory and field experiments. However, the overall results showed that these alternative control methods can be effective in reducing the number of larvae as well as severity of feeding damage symptoms.

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Chapter 3

Conclusions and recommendations

3.1 Conclusions

The use of synthetic insecticides against FAW does not always provide effective control or reduced yield losses, partly due to incorrect application methods (Bateman *et al.* 2021, van den Berg *et al.* 2021). This poor field-performance of chemical control applications has a significant negative effect on crop yield, especially in smallholder farming systems in Africa. This is because insecticide application recommendations or procedures are not always followed (Tepa-Yotto *et al.* 2022), and as a result, FAW have developed resistance against a broad spectrum of insecticides (Carvalho *et al.* 2013). However, several smallholder farmers in Africa responded to FAW infestations with synthetic insecticides (Houngbo *et al.* 2020, Sisay *et al.* 2019a), although they are not always efficient (Sisay *et al.* 2019a). The development of insecticide resistance by FAW have made the use of insecticides unsustainable in many parts of the world, and the cost of these products also puts its use beyond that of most smallholder farmers in Africa. There is therefore, a need for the development of alternative control methods (Tepa-Yotto *et al.* 2022) which are sustainable and safe to people and the environment.

Alternative control methods such as the whorl applications of ash, soil and sand are used by smallholder farmers and may provide safe and sustainable management of FAW if they are effective. These materials are easily available for smallholder farmers (Yigezu and Wakgari 2020) and they are natural, which means they can successfully provide sustainable control of FAW larvae without the development of resistance. This study showed that, although results of the field trials and laboratory experiments were variable, the application of ash and soil into plant whorls as curative treatments, may result in significant reductions in survival of 3rd-instar larvae. Furthermore, non-preference by larvae for ash and soil-treated leaf tissue resulted in the abandonment of leaf tissue. Under field conditions, this may lead to increased exposure of larvae to natural enemies, which may contribute further to suppression of pest populations.

These results therefore provide hope for the livelihoods of millions of smallholder farmers who are dependent on maize as a staple crop (Banson *et al.* 2019, Chimweta *et al.* 2019, Sylvain *et al.* 2015). Moreover, the dependence on, and inappropriate application of insecticides by smallholder farmers (Tepa-Yotto *et al.* 2022) can be reduced by using ash and soil as alternatives to synthetic insecticides. This could therefore minimize the rate and development of insecticide resistance on FAW populations. Studies have shown that majority of smallholder farmers do not have access to effective insecticides because of high costs (Bateman *et al.* 2021) and, as a result, they use ineffective low-cost insecticides. Smallholder farmers can therefore apply ash and soil instead of ineffective insecticides, and through this, reduce their exposure to health risks posed by applying insecticides without proper PPEs.

FAW infestation during the early growth stages of maize, particularly during seedling stages can result in complete defoliation and crop loss (van den Berg *et al.* 2021). Protecting plants during early growth stages has been reported to significantly reduce yield loss in contrast to protection during late plant growth stages (van den Berg *et al.* 2021). As indicated in the results of the present study, soil and ash applied as preventative control measures against 1^{st}-instar FAW larvae can reduce survival as well as the severity of leaf feeding damage symptoms. Therefore, soil and ash can be used as a preventative measure to provide protection of maize during early growth stages (seedling emergence to early whorl stage) during which plants are highly susceptible to FAW. Application of ash from the V1-growth stage at weekly intervals has been reported to reduce FAW feeding damage (Lyle *et al.*, 2021). The invasiveness (Sokame *et al.* 2020) and difficulties of FAW control (Bateman *et al.* 2018, Matova *et al.* 2020) continues to point to the need for promoting alternative control methods that directly target the larvae inside the whorls of plants. Fall armyworm larvae feed inside the whorls of plants which makes it difficult to control (Bateman *et al.* 2018, Matova *et al.* 2020). This cryptic feeding behaviour makes it difficult for insecticides applications to reach the larvae inside the whorl, especially when using contact insecticides (Bateman *et al.* 2018). Whorl applications of soil and ash has direct contact effects on FAW larvae since it directly targets the larvae inside the whorl. Therefore, soil and ash can contribute to sustainable, environmentally friendly control methods that can be used by smallholder farmers to control this pest. Furthermore, soil and ash were also found to provide some level of control of different larval stages (1^{st}, 3^{rd} and 5^{th}-instar) of FAW.

Both wet and dry ash effectively controlled 1^{st}-instar FAW larvae within 48 hours of treatment in laboratory studies by obtaining high levels of larval mortality. Soil, wet ash and dry ash reduced larval survival on 3^{rd}-instars and feeding damage on 1^{st}-instars under field conditions within five days of treatment. However, wet ash should be used with caution because it was observed to burn the leaves after rain that occurred during the field experiments.

3.2 Recommendations

Future research should also evaluate the effect of ash and soil applications on large larvae (5^{th}-instars) under field conditions, since, in this study, experiments on 5^{th}-instar were only conducted under laboratory conditions. In this study, only one application of ash or soil was made to control FAW larvae, and assessments conducted after a period of five days. It is therefore recommended that more studies be conducted to determine the effect that two or three applications during a cropping season may have on pest damage. On-farm trials in which the possible effects of different intervals between whorl applications of ash and soil should also be conducted. Since cannibalism, larval dispersal and movement off from plants is characteristic of FAW, it is recommended that field trials be conducted under natural infestations rather than artificial inoculation in order to obtain accurate results in terms of larval mortality.

Some studies have indicated that these alternative control methods are not effective, which is contrary to some of the results of this study. Therefore, it is recommended that further research be conducted to assess the efficacy of different soil types and wood species, and different ''dosage rates'' (volumes) of ash and soil to apply at different plant and larval growth stages in order to understand the differences in the levels of control achieved by whorl applications of ash and soil, in different regions. Environmental factors such as rain should be taken into consideration in future studies because the rain can also affect the efficacy of these alternative control methods by washing ash, soil and sand out of maize whorls.

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