The efficacy of selected herbicide-adjuvant mixtures for the control of Roundup Ready (glyphosate-resistant) volunteer maize

by

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Declaration

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Summary

Roundup Ready (glyphosate-resistant) volunteer maize is a common occurrence in any production system where maize is planted. Volunteer maize is a result of maize seeds or ears that escaped harvesting in the previous year or season. Clethodim, quizalofop-P-tefuryl and glufosinate-ammonium were applied in combination with five adjuvants in order to establish the most effective herbicide-adjuvant combination for each of the three herbicides. A surfactant, penetrant, humectant, salt adjuvant and drift control agent was combined with the three herbicides in various combinations. Necrosis, stunting and mortality was assessed to determine the efficacy of the herbicide-adjuvant combinations. Two trials were executed to investigate the efficacy of the herbicide-adjuvant combinations. The first was a deposition trial where the combinations were applied at four different water volumes to investigate the effect of water volume on the efficacy of the herbicide-adjuvant mixtures. The second was an efficacy trial where the combinations were applied at the water volume as prescribed by the product labels. The deposition and efficacy trials were duplicated at two trial sites. An increase in water volume generally led to an increase in efficacy. The penetrant and humectant proved most successful with quizalofop-P-tefuryl. The penetrant increased the efficacy of clethodim significantly whereas the salt adjuvant proved most successful to combine with glufosinate-ammonium.

Opsomming

Roundup Ready (glifosaat weerstandbiedende) opslagmielies is ‘n algemene probleem in produksiestelsels waarin mielies geplant word. Opslagmielies is die gevolg van mieliekoppe of –saad wat nie in die vorige seisoen of jaar ingesamel is nie. Clethodim, quizalofop-P-tefuriel en glufosinaat ammonium is in kombinasie met vyf verskillende bymiddels op opslagmielies toegedien om vas te stel wat die mees doeltreffende onkruiddoder-bymiddel kombinasie is vir elk van die drie onkruiddoders. ‘n Benatter, penetreermiddel, herbenatter, soutbyvoegmiddel en neerslaghulpmiddel is in verskeie kombinasies met die onkruiddoders gemeng. Nekrose, verdwerging en mortaliteit is ge-evalueer om die doeltreffendheid van die onkruiddoder/bymiddel kombinasies te bepaal. Twee proewe is uitgevoer om die doeltreffendheid van die onkruiddoder/bymiddel kombinasies te ondersoek. Die eerste was ‘n bedekkingsvlaktoets waar die kombinasies teen vier verskillende watervolumes toegedien is om die effek van watervolume op die doeltreffendheid van die onkduiddoder/bymiddel mengsels te bepaal. Die tweede was ‘n doeltreffendheidstoets waar die kombinasies toegedien is teen die watervolume soos voorgeskryf op die etikette van die produkte. Die bedekkingsvlak- en doeltreffendheidstoetse is op twee lokaliteite herhaal. Toename in watervolume het oor die algemeen gelei tot verbeterde doeltreffendheid. Die penetreermiddel en herbenatter was die beste bymiddel met quizalofop-P-tefuriel. Die penetreermiddel het die doeltreffendheid van clethodim vebeter terwyl die soutbyvoegmiddel die beste bymiddel was om te gebruik saam met glufosinaat ammonium.

This thesis is dedicated to:

To all the men and women who dedicate themselves to the Agriculture industry in any way, shape or form. Thank you for feeding a nation.

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Preface

This thesis is presented as a compilation of 7 chapters.

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Chapter 1: General introduction and project aims

1.1 Volunteer plants

A volunteer plant is defined as a plant growing in an area where it was not planted, as if by natural regeneration (Soltani et al. 2006). Volunteer plants are generally welcomed because of the habitat they create and the contribution they make towards healthy ecosystems and biodiversity in an area (Soltani et al 2006). These plants are also undesired in agriculture where they are considered weeds (Soltani et al. 2006).

Volunteer maize is a result of maize seeds or ears that escaped harvesting in the previous year or season. Volunteer maize is also common in a maize replant situation following a failed stand (Chahal et al. 2016).

Volunteer maize plants are therefore maize plants that grow in fields where they established by means of natural regeneration and are considered weeds. The impacts of these weeds are explained in Chapter 2 of this thesis. In South Africa these weeds are a common occurrence in any situation where maize was planted, and the effective control of these weeds is a study worth pursuing.

1.1.1 Frequency of occurrence

The estimated area on which maize was planted in South Africa in 2018 was 2 607 000 hectares (Department of Agriculture 2018). That vast area yielded approximately 12 783 000 tons of maize (Department of Agriculture 2018). From these numbers the conclusion can be made that maize is a very common and frequently planted crop.

Due to the process of volunteer maize establishment an assumption may be made that due to the frequent occurrence of maize as a crop, volunteer maize will also be a frequent occurrence in South Africa.

1.1.2 Roundup Ready volunteer maize

Roundup Ready crops are crops which are genetically modified (GM) to resist the effects of glyphosate when the herbicide is applied (Owen and Zelaya 2005). The ability of the glyphosate-resistant plants to remain unharmed during post-emergence applications of glyphosate led to the large-scale adoption of these crop varieties (Owen and Zelaya 2005). Roundup Ready crop varieties accounted for more than 90% of all cotton, soybean and maize crops in the United States of America by 2014 (Duke 2017).

Roundup Ready volunteer maize is thus the phenomenon where volunteer maize exhibits a tolerance towards glyphosate. The tolerance towards glyphosate only further exacerbates the impact of these weeds because glyphosate is the most widely used herbicide in the world, including pre-plant situations (Owen 2008).

1.1.3 Impact of Roundup Ready volunteer maize

Glyphosate-resistant volunteer maize has far-reaching effects such as direct competition with crops for sunlight, nutrients and water. Various sources report a decrease in crop yields due to the presence of glyphosate-resistant volunteer maize (Andersen et al. 1982, Jeschke and Doerge 2008, Alms et al 2016).

Glyphosate-resistant volunteer maize also has indirect impacts. These impacts range from selecting for insect resistance to the *Bt*-gene to selecting for widespread resistance to glyphosate in various weed species (Duke 2017).

1.2 Focus of the study

The focus of this study was to determine whether the addition of agrochemical adjuvants would improve the efficacy of clethodim, quizalofop-P-tefuryl and glufosinate-ammonium to control glyphosate-resistant volunteer maize. A multitude of factors affect the efficacy of these herbicides and this study aims to establish which herbicide-adjuvant mixtures remains the most effective in a variety of situations. These factors range from weed growth stages during application to different dosage rates of the herbicide applied (Mucheri 2016).

1.3 Value of the study

This study carries great value as it strives to answer frequently asked questions about volunteer maize, the impacts of adjuvants and the influences of deposition on the effects of different herbicides to control glyphosate-resistant volunteer maize. The study further strives to uncover the most effective herbicide-adjuvant mixtures for controlling volunteer maize.

1.4 Aims and objectives

There are a variety of aims and objectives for this study but the main aim throughout the study was to determine the most effective herbicide-adjuvant mixture to control glyphosate-resistant volunteer maize. The three mentioned herbicides were applied, evaluated and analysed independently of one another, and was not compared to each other, instead the aim was to determine which specific herbicide-adjuvant mixture proved the most effective for each herbicide. This was done because all three of the herbicides warrants its own niche and use in a spray programme.

1.4.1 Aim

The aim for this study was to determine the efficacy of selected herbicide-adjuvant mixtures for the control of glyphosate-resistant volunteer maize.

1.4.2 Objectives

The first objective was to determine which clethodim-, quizalofop-P-tefuryl-, and glufosinate-ammomiun adjuvant mixtures were the most effective in causing stunting, necrosis and mortality of glyphosate-resistant volunteer maize.

The second objective was to establish whether a change in water volume during application affected the efficacy of the herbicide-adjuvant mixtures in causing stunting, necrosis and mortality of glyphosate-resistant volunteer maize.

The third objective was to determine whether a change in water volume during application affected the deposition of mixtures. Various claims are made that adjuvants improve deposition and it was an objective of this study to confirm or deny these claims.

1.5 REFERENCES

Alms J, Moeching M, Vos D, Clay S. 2016. Yield loss and management of volunteer corn in soybean. *Weed Technology 30: 254-262.*

Andersen RN, Ford JH, Leuschen WE. 1982. Controlling volunteer corn (*Zea mays*) in soybeans (*Glycine max*) with diclofop and glyphosate. *Weed Science* 30: 132-136.

Chahal PS, Jha P, Jackson-Ziems T, Wright R, Jhala AJ. 2016. Glyphosate-resistant volunteer maize (*Zea mays* L.): impact and management. In: Travlos IS, Bilalis D, Chachalis D (eds), *Weed and Pest Control.* Lincoln: Nova Science Publishers. pp 83-94.

Department of Agriculture, Forestry and Fisheries. 2018. Abstract of agricultural statistics. Available at 1 [accessed 20 September 2019].

Duke SO. 2017. The history and current status of glyphosate. *Pest Management Science* 74: 1027-1034.

Jeschke M, Doerge T. 2008. Managing volunteer corn in cornfields. *Crop Insights* 18: 1-4.

Mucheri T. 2016. The efficacy of glufosinate-ammonium on ryegrass as influenced by different plant growth stages and different temperatures.MSc thesis, Stellenbosch University, South Africa.

Owen M. 2008. Weed species shifts in glyphosate-resistant crops. *Pest Management Science* 64: 377-387.

Owen M, Zelaya I. 2005. Herbicide-resistant crops and weed resistance to herbicides. *Pest Management Science* 61: 301-311.

Soltani N, Shropshire C, Sikkema, P. 2006. Control of glyphosate-tolerant maize (*Zea mays*) in glyphosate-tolerant soybean (*Glycine max*). *Crop Protection* 25: 178-181.

Chapter 2: Literature review

2.1 Volunteer maize:

Volunteer maize is a result of maize seeds or ears that was not harvested the previous year or season. Volunteer maize is also common in a maize re-plant situation following a failed stand (Chahal et al. 2016). Factors responsible for kernel and ear loss of maize is also responsible for the presence of volunteer maize. Insect damage, weather damage, harvesting problems and poor stalk quality all contribute to kernel and ear loss of maize and therefore may also be blamed for the presence of volunteer maize (Chahal et al. 2016).

2.1.1 Roundup Ready volunteer maize

Roundup Ready crops are crops which are genetically modified (GM) to tolerate the effects of glyphosate herbicides when the herbicide is applied (Owen and Zelaya 2005). This tolerance is made possible due to the encoding of the glyphosate-resistant enzyme known as CP4 EPSP synthase (Funke et al. 2006). The ability of the glyphosate-resistant plants to remain unharmed during post-emergence applications of glyphosate has led to the large-scale adoption of these crop varieties (Owen and Zelaya 2005). Roundup Ready crop varieties accounted for more than 90% of all soybeans, cotton and maize planted in the United States of America in 2014 (Duke 2017).

Roundup Ready volunteer maize can therefore be viewed as volunteer maize that possesses the glyphosate-resistant enzyme CP4 EPSP synthase. The tolerance of Roundup Ready volunteer maize plants to glyphosate has far reaching effects and only adds to the impacts of these weeds on agricultural systems (Gressel 2010).

2.1.2 Impact of volunteer maize`

Volunteer maize has a variety of far reaching impacts. These impacts vary from being the direct cause of yield loss to the indirect selection of resistance in insect species.

As one may expect, volunteer maize competes with the crop for water, light and nutrients in the same way as any other weed species will (Jeschke and Doerge 2008). Volunteer maize plants with a density of 0.5 to 4 plants per square meter accounted for yield losses ranging from 1.5 to 13% in a hybrid maize stand (Jeschke and Doerge 2008). A volunteer maize density of 0.4 maize plants for every straight-line meter had a yield loss impact of 14 to 49% in soybeans (Andersen at al. 1982). One volunteer maize plant, per square meter, was responsible for a yield loss of up to 19.3% in dry beans (Sbatella et al. 2016).

Data from two consecutive years of study showed that volunteer maize at varying densities impacted the yield of soybeans (Alms et al. 2016). A plant density of less than 0.3 volunteer maize plants per square meter was responsible for a 9% yield loss (Alms et al. 2016). Plant densities higher than 0.3 volunteer maize plants per square meter was responsible for a 25 to 29 % yield loss in soybeans.

Piasecki et al. (2017) observed that volunteer maize plants influenced the individual yield components of soybean plants. A decrease was observed in the shoot dry weight, mean number of grains and the thousand kernel mass due to the presence of volunteer maize (Piasecki et al. 2017).

Volunteer maize has an abundance of impacts and effects on yields due to direct competition, as has been proven by various sources. These weed plants also have the potential to impact crops and yields indirectly (Marquardt et al. 2013).

During periods of fallow rotation volunteer maize plants exhibit the ability to reduce soil water by 2.45 cm for every 0.62 volunteer plants per square meter (Marquardt et al. 2013). The overall reduction in available soil water reduced wheat yields, that were planted after the fallow period, by up to 63 kilograms per hectare (Marquardt et al. 2013).

Marquardt et al. (2013) further states that F2 generations of maize cultivars that contained the *Bacillus thuringiensis (Bt)* entomopathogenic bacteria exhibits this bacterium and its toxins but at a lower sub-lethal dose. The Bt gene is a gene that contains the highly specific *Bacillus thuringiensis* entomopathogenic bacteria that is used in maize for the control of insect pests (Hilbeck and Schmidt 2006). A major concern arises if insects are exposed to a sub-lethal dose of the Bt gene and its toxins, due to the likelihood of resistance evolving from this sub-lethal exposure (Marquardt et al. 2013).

Krupke et al. (2009) tested the F2 generations of maize plants that contained the Bt gene. The F2 generation, volunteer maize plants, were sourced from various soybean fields. The results from the study caused that 65% of the tested population contained the Bt gene. Evaluations of feeding incidence and severity caused by the Western Corn Rootworm (WCR) caused that there were no significant differences between volunteer maize containing the Bt gene and volunteer plants that did not contain the Bt gene (Krupke et al. 2009).

From the above results Krupke et al. (2009) made the conclusion that the WCR fed on the F2 generation with the Bt gene present but the gene had no effect in controlling the insect pest. These results confirm the findings of Marquardt et al. (2013) that insects are being exposed to sub lethal doses of the Bt gene due to the presence of volunteer maize that contains the Bt gene. The exposure of insects to the sub-lethal doses of the Bt gene will eventually lead to an insect population that evolves resistance to this gene (Krupke et al. 2009, Sbatella et al. 2016).

The main reason for growers to follow a rotational crop program is to disrupt pest cycles (Krupke et al. 2009, Sbatella et al. 2016).The presence of volunteer maize plants will act as a host for pests to survive during crop cycles when maize is not being planted, thereby debilitating the use of crop rotation systems (Deen et al. 2006).

There are various diseases and insects that make use of volunteer crop residues to over-winter or to survive during years where maize is not planted. Northern corn leaf blight (*Exserohilum turcicum*) is known to cause lodging during harvesting and is one of the most common diseases to survive on volunteer maize or crop residues (Chahal et al. 2016). Grey leaf spot (*Cercospora zeae*) is also commonly found on volunteer maize (Chahal et al. 2016). Grey leaf spot (GLS) is considered the most devastating and yield-limiting disease of maize in southern Africa (Meisel et al. 2009). The presence of volunteer maize supports the survival and overwintering of the above-mentioned diseases which in turn affects the yield of following seasons.

Busseola fusca, the African Maize Stalk Borer, is one of the most common insect pests of maize and other grass crops across the whole of Africa (Harris and Nwanze 1992). These pests depend on crop residues and alternative hosts to survive winters and in times when maize is absent. The primary cultural control methods for these insect pests are destruction of crop residues and crop rotation (Harris and Zwane 1992). The presence of volunteer maize completely destroys the purpose and efficacy of these cultural control methods which means growers are left with only one option, to apply an additional insecticidal spray program to control these pests during times when maize is not even being planted (Harris and Zwane 1992).

It is clear from the literature that volunteer maize plants pose many threats, from direct competition to the selection for insect resistance. The question then arises: how do we control these weeds?

2.1.4 Control of volunteer maize

There are few options available for the control of volunteer maize. These options are no-tillage, the correct combine harvester settings, different timings of tillage, crop rotation, pre-emergence herbicides and post-emergence herbicides.

The employment of no-tillage systems will expose the volunteer maize seed to predation and lower temperatures in winter (Alms et al. 2016). No-tillage is not a 100% reliable approach due to the volunteer maize seed being exposed to favourable conditions. Whereas in a conventional tillage system the seed will be buried deep within the seedbed (Alms et al. 2016).

The correct combine setup for the terrain is of paramount importance when the purpose is to minimise seed loss to prevent volunteer maize from establishing (Jeschke and Doerge 2008). Harvesters that are currently available, however, is not yet refined enough to use as a tool to prevent volunteer maize from establishing (Jeschke and Doerge 2008).

Tillage timings, when used effectively, along with post emergence herbicides, is a very effective method of controlling volunteer maize (Alms et al. 2016). Tilling a field shortly after harvesting will bury seed that was lost during harvesting. Tillage of the field again a few weeks before planting will expose those viable seeds to the upper layer of the seedbed and encourage germination. The plants that germinate can then be targeted with a post emergence herbicide to control the volunteer maize population before planting commences (Alms et al. 2016).

Crop rotation is a very valuable control method for volunteer maize if an abundance of volunteer maize is present in other grass crops (Owen 2008). A crop rotation with a broadleaf crop will then present the opportunity to control volunteer maize with a selective post emergence herbicide (Owen 2008).

Pre-emergence herbicides do not control volunteer maize consistently (Chahal et al. 2016). When conditions are perfect for the application of pre-emergence herbicides, they do control volunteer maize effectively, but growers rarely employ this control strategy due to the uncertainty of results and the economic impact that this uncertainty may have (Chahal et al. 2014).

The strategy that is the most successful for the control of volunteer maize is the application of post-emergence herbicides (Alms et al. 2016). Post-emergence herbicides are applied across the world for the control of these weeds and it is the only strategy that can be integrated with multiple control strategies (Chahal et al. 2016). Due to the efficacy of post-emergence herbicides to control, specifically glyphosate-resistant volunteer maize, this study focusses on selected herbicides to control these weeds and how to improve their efficacy with the addition of a variety of adjuvants.

2.2 Herbicides

2.2.1 Why not glyphosate?

Glyphosate is the most used herbicide in the world (Owen 2008). The uses for glyphosate vary from commercial agricultural uses to household control of problematic weeds (Owen 2008). The adoption of glyphosate resistant variant crops has only accelerated the dependency on glyphosate to control weeds. For the control of glyphosate-resistant volunteer maize however, glyphosate is not an option and alternative herbicides have to be considered.

2.2.2 Herbicide mode of action

The mode of action of an herbicide is the way an herbicide acts on the metabolic function of plants or disrupts the energy transfer in plant cells (Duke 1990). When herbicides and plants interact, it is the mode of action of herbicides that enable herbicides to disrupt the physiological processes of weeds and control weed populations (Duke 1990).

Mode of actions is also responsible for the ability to apply certain herbicides on crops without harming the crop on which it is sprayed. The mode of action therefore influences which herbicides can be applied to control glyphosate-resistant volunteer maize without damaging the crop in which it is present (Retzinger and Mallory-Smith 1997).

In this study, two groups of herbicides were investigated for the control of glyphosate-resistant volunteer maize. These groups are group A and group H (Retzinger and Mallory-Smith 1997). Group A herbicides are inhibitors of acetyl CoA carboxylase ACCase which disrupts lipid synthesis used to form cell membranes (Baumann et al. 2008). Thus, Group A herbicides disrupts the formation of cell membranes which causes death in the plant (Baumann et al. 2008). Clethodim and quizalofop-p-tefuryl are two group A herbicides that were tested in this study (Retzinger and Mallory-Smith 1997). Group H herbicides are inhibitors of glutamine synthase and the only herbicide in group H is glufosinate-ammonium (Retzinger and Mallory-Smith 1997).

Group A herbicides are selective grass herbicides and will not harm, damage or effect broadleaf plants and is therefore safe to use within any broadleaf crop (e.g. soybeans) to control glyphosate-resistant volunteer maize (Duke 1990). Group H herbicides are non-selective herbicides and will therefore damage any plant it is applied on (Duke 1990). The question then remains how to incorporate these herbicides into a chemical control programme to effectively control glyphosate-resistant volunteer maize without damaging the crop in which these weeds are present.

2.2.3 Alternative herbicides and their uses

Three alternative herbicides to glyphosate have been identified to control glyphosate-resistant volunteer maize, based on their mode of action. As already mentioned, these three herbicides are clethodim, quizalofop-P-tefuryl and glufosinate-ammonium and each of these herbicides will be discussed separately to explain why these herbicides were identified.

2.2.3.1 Clethodim and quizalofop-P-tefuryl

ACCase inhibitors are the most popular herbicide group for the control of glyphosate-resistant volunteer maize (Marquardt and Johnson 2013). For this reason, two of these herbicides have been selected to test efficacy for the control of these weeds.

Clethodim and quizalofop-P-tefuryl do not differ radically from one another (Chahal and Jhala 2015). Clethodim has a waiting period of seven days after application before any grass crop can be planted (Chahal and Jhala 2015). Clethodim is therefore not an option to control glyphosate-resistant volunteer maize in systems where grass crops follow one another. Quizalofop-P-tefuryl is an ACCase inhibitor with a waiting period of 1 day after application before planting can commence which means it is a more viable option for the control of glyphosate-resistant volunteer maize in rotational crop systems where grass crops follow one another (Marquardt and Johnson 2013).

ACCase inhibitors will not damage broadleaf plants and is therefore the only option available to growers when glyphosate-resistant volunteer maize occurs in an already established broadleaf crop field (Chahal and Jhala 2015). Due to these two herbicides' specific mode of action and their residual characteristics these two herbicides will be used in different ways to control glyphosate-resistant volunteer maize.

Clethodim has effective compatibility with glyphosate (Marquardt and Johnson 2013). This makes clethodim a very effective herbicide option when glyphosate resistant volunteer maize and broadleaf weeds are present in glyphosate-resistant broadleaf crops, e.g. soybeans. Clethodim is then added to the tank mix along with glyphosate to control glyphosate-resistant volunteer maize, grass weeds and broadleaf weeds (Marquardt and Johnson 2013). Pertile et al. (2018) revealed that clethodim mixed with glyphosate still managed to obtain 85% control of volunteer maize. This ability ensures that clethodim is a popular option for growers because effectively it means the farmer must only spray once which has enormous economic benefits (Marquardt and Johnson 2013).

Quizalofop-P-tefuryl is an attractive option as a pre-plant application in a field where glyphosate-resistant volunteer maize is present (Chahal et al. 2014). This is because of the short residual activity of this herbicide compared to clethodim (Baumann et al. 2008). Quizalofop-P-tefuryl can also be sprayed in a field of broadleaf crops to control glyphosate-resistant volunteer maize and other grass weeds that are present (Chahal et al. 2014). Quizalofop-P-tefuryl also has compatibility with glyphosate but cases have been reported where the quizalofop-P-tefuryl efficacy decreases when tank mixed with glyphosate (Gressel 2010).

2.2.3.2 Glufosinate-ammonium

Glufosinate-ammonium is a non-selective herbicide and will damage or kill any plant that it gets into contact with (Duke 1990, Buamann et al. 2008, Carbonari et al. 2016). This characteristic of glufosinate-ammonium then begs the question: why would one apply this herbicide and how does this herbicide fit into a chemical control programme for glyphosate-resistant volunteer maize?

Glufosinate-ammonium cannot be applied as a post emergence herbicide because the herbicide will damage or even kill the crop. Glufosinate-ammonium is very effective as a pre-plant herbicide in a field where glyphosate-resistant volunteer maize is present (Chahal and Jhala 2015). Quizalofop-P-tefuryl is also a favoured herbicide for the control of glyphosate-resistant volunteer maize as a pre-plant application so why then test two herbicides for the same purpose?

The constant use of the same herbicide will lead to target-site resistance (Yuan et al. 2006). According to Abbas et al. (2017) ACCase inhibitors are one of the herbicide groups that are most susceptible to herbicide resistance. Glufosinate-ammonium fits into a chemical programme to control glyphosate-resistant volunteer maize by acting as a substitute to ACCase inhibitors to prevent or delay herbicide resistance from occurring for this group of herbicides (Chahal and Jhala 2015). Glufosinate-ammonium is also a very effective option for the control of a variety of weed species making it the ideal herbicide to control glyphosate-resistant volunteer maize in fields where there are dense weed populations (Chahal and Jhala 2015). Quizalofop-P-tefuryl is not a viable option for this purpose because the ACCase inhibitors will only control grass weeds and the application of an additional ACCase inhibiting herbicide will only further exacerbate the potential for herbicide resistance (Abbas et al. 2017).

The versatility of glufosinate-ammonium to control various weeds and the role it plays in the prevention of herbicide-resistance warrants the testing of this herbicide for the control of glyphosate-resistant volunteer maize.

2.2.4 Factors influencing herbicide efficacy

There are various factors influencing herbicide efficacy. When the focus is placed on volunteer maize the amount of influences on herbicide efficacy drastically decreases. When controlling volunteer maize, there are three factors that influence efficacy.

The first of these major influences has to do with plant density and penetration or coverage of the herbicide. When an herbicide is applied to a dense stand of glyphosate volunteer maize the efficacy of herbicides to control these weeds decreases (Alms et al. 2016). The efficacy decreases in higher densities simply because there is not a large enough amount of herbicide that makes effective contact with enough of the target area to evoke a plant response to effectively control glyphosate-resistant volunteer maize (Deen et al. 2006).

Another major influence on the efficacy of herbicides to control glyphosate-resistant volunteer maize is the size of the volunteer maize. ACCase inhibitors act by being transported to the growth point of the volunteer maize plant and affect the growth of the volunteer maize plant (Alms et al. 2016). Larger volunteer maize plants possess a higher metabolic rate and can counter the effect of ACCase inhibitors at a faster rate (Chahal et al. 2014). Chahal et al. (2014) proved this theory by concluding that maize plants at the 2-3 leaf stage were more susceptible to ACCase inhibitors than larger maize plants. Maize plants shorter than 30 cm were also more effectively controlled with clethodim than maize plants larger than 90 cm (Marquardt and Johnson 2013). For glufosinate-ammonium the opposite was true where an increase in efficacy was observed with an increase in ryegrass (*Lolium spp.)* growth stage (Mucheri 2016).

The last factor contributing to the efficacy of herbicides to control glyphosate-resistant volunteer maize are climatic conditions. Glufosinate-ammonium is less effective when low temperatures and low relative humidity conditions are prevailing (Kumaratilake and Preston 2005). The same high metabolic rate that can counter the effect of clethodim, can also have an effect when the metabolic rate is too low. An ACCase inhibitor is only effective when the weed it is applied to is actively growing (Marquardt and Johnson 2013). This allows for maximum translocation of the herbicide and greater effect on the growth point. If a plant is growing at a low metabolic rate due to lower temperatures, the efficacy of ACCase inhibitors will decline due to a decrease in translocation and minimal influence on the growth point of the volunteer maize plant (Alms et al. 2016).

The three major influences on the efficacy of herbicides plays a substantial role in the control of glyphosate volunteer maize. One way to counter these influences is by adding an adjuvant to the herbicide tank mix to improve efficacy.

2.3 Adjuvants

Adjuvants are added to herbicides to improve efficacy (Green 1992). Various herbicide manufacturers prescribe adjuvants to be added to their formulation or to be tank mixed to improve efficacy (Green 1992). Adjuvants are also prescribed to reduce dosages and as many as two adjuvants may be added to a tank mix due to the presence of a spectrum of weeds and prevailing unfavourable environmental conditions (Green 1992).

2.3.1 Adjuvant overview

Hazen (2000) defined an adjuvant as a material added to a tank mix to aid or modify the action of an agrichemical, or the physical properties of the mixture. An adjuvant is therefore something that is added to a spray solution to increase the efficiency of an active ingredient (Zollinger 2012). These materials may be formulated with the herbicide or added to the herbicide in a tank mixture to create a spray solution (Curran et al. 1999).

Adjuvants can be grouped into two basic groups. The first are adjuvants that alter the physical characteristics of the spray solution and therefore the physical characteristics of the herbicide (Jordan et al. 2011). The second group are adjuvants that contribute to the increased biological action of the herbicide, thereby increasing the efficacy of herbicides (Hazen, 2000). The first group of adjuvants are known as utility adjuvants and the second group is known as activator adjuvants (Curran et al. 1999). Both these groups of adjuvants increase the efficacy of herbicides by creating a synergism.

2.3.2 Synergism

Synergism of agrichemicals is the reason for improved efficacy when an adjuvant is added to an herbicide (Rao 2000). Synergism can be explained as the enhanced penetration, translocation or biological action of an herbicide due to the presence of an additional chemical compound (Rao 2000). Adjuvants play the role of the added chemical and the result is a synergism that increases the efficacy of herbicides (Curran et al. 1999).

Figure 1 portrays the mechanism of synergism when an adjuvant is added to an herbicide to improve efficacy.

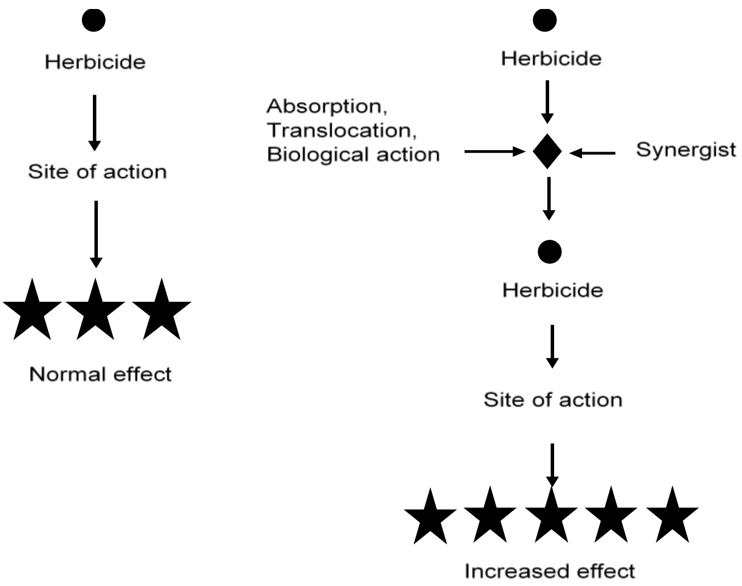


Figure 1: Synergism mechanism when adjuvant is added to an herbicide (Rao 2000)

2.3.3 Utility adjuvants

Utility adjuvants are added to spray mixtures with the intention to aid in the improvement of the application process and do not directly influence the efficacy of herbicides (McMullan, 2000). Utility adjuvants indirectly increases the efficacy of herbicides by improving the spray application (Xu et al. 2010).

Utility adjuvants can be subdivided into five primary utility adjuvants and three secondary utility adjuvants (McMullan, 2000). The primary utility adjuvants include drift control agents, water conditioning agents, deposition agents, compatibility agents and defoaming agents. The secondary utility adjuvants include colorants, buffering agents and acidifying agents (McMullan, 2000).

In this study one primary utility adjuvant was tested with herbicides to evaluate the effect the adjuvant has on the efficacy of herbicides. The adjuvant consists of vegetable oils and polyoxy ethylene fatty acid ester designed to increase deposition of herbicides. Deposition adjuvants improves the deposition of herbicides by increasing the amount of herbicide that are deposited directly on the target area (Lan et al. 2008). Deposition agents can also indirectly increase deposition by increasing the uniformity of herbicide deposition on the plant surface (Xu et al. 2010). The deposition agent used in this thesis is the Villa Crop Protection product Interlock™.

2.3.4 Activator adjuvants

Activator adjuvants are added to herbicides to directly influence the efficacy of herbicides (Penner, 2000). Activator adjuvants may be added directly to the herbicide formulation or may be added to create a tank mixture (Penner, 2000). Activator adjuvants efficacy is not only a function of the adjuvant but also of the herbicide, prevailing environmental conditions and the specific weed spectrum it is applied to (Penner, 2000). These adjuvants therefore directly affect the efficacy of the herbicide (Penner, 2000).

Activator adjuvants are subdivided into wetter-spreader adjuvants (surfactants), sticker adjuvants, humectants, penetration agents, translocation agents and herbicide modifiers (Hazen, 2000). In this study three activator adjuvants were tested to evaluate and analyse their influence on the efficacy of herbicides. The first was a surfactant/oil adjuvant combination that consists of a polyether-polymethylsiloxane- copolymer combined with a vegetable oil to serve as wetter-spreader. This adjuvant increases the efficacy of herbicides by creating a less spherical droplet which in turn leads to a larger surface area covered by one droplet. An increased surface area directly leads to an increase in herbicide activity because a larger quantity of the herbicide active ingredient encounters the target area (Czarnota and Thomas 2010).

The second activator adjuvant in this study was a surfactant/fertilizer combination to serve as humectant and a wetting and spreading agent. Humectants increases herbicidal activity and efficacy by keeping the solution in a liquid form (Tu and Randall 2003). Humectants make this possible by extracting moisture from the surrounding atmosphere and ensuring a higher humidity which leads to a decreased rate of drying off (Xu et al. 2010).

A third activator adjuvant was tested which belongs to the penetration agents. The adjuvant consists of a high surfactant oil concentrate (HSOC) methylated seed oil. These adjuvants increase herbicide efficacy by disrupting or softening the cuticular waxes that are present on plant leaves, thereby aiding the penetration and absorption of herbicides (Jordan et al. 2011).

The fourth activator adjuvant tested was a liquid formulation of ammonium sulphate/surfactant/humectant combination designed to act as a salt adjuvant. A salt adjuvant increases the efficacy of herbicides by altering or minimising ionic interactions in spray solutions that would have reduced herbicide efficacy if left unaltered (Travlos et al. 2017). Class Act NG™ will serve as the water conditioning agent ammonium sulphate.

The main aim of this thesis was to determine the efficacy of the above discussed herbicide and adjuvants, in various combinations, for controlling glyphosate-resistant volunteer maize. Quizalofop-P-tefuryl and clethodim was chosen due to the fact that ACCase inhibitors are the most widely used group of herbicides to control volunteer maize. The ACCase inhibitors is also one of the herbicide groups which are most susceptible to the development of herbicide resistance (Chahal and Jhala 2015). Due to this, glufosinate-ammonium was also tested as a substitute to the ACCase inhibitors to avoid herbicide resistance from occurring.

The five adjuvants were selected because they represent both of the two main types of adjuvants, activator-and utility adjuvants. The adjuvants selected are the adjuvants that are most widely prescribed to use with all three herbicides and therefore they were selected.

2.4 References

Abbas T, Nadeem MA, Tanveer A, Ali HH, Matloob A. 2017. Evaluation and management of acetyl-CoA carboxylase inhibitor resistant littleseed canarygrass (*Phalaris minor*) in Pakistan. *Archives of Agronomy and Soil Science* 17:1-10.

Alms J, Moeching M, Vos D, Clay S. 2016. Yield loss and management of volunteer corn in soybean. *Weed Technology* 30: 254-262.

Andersen RN, Ford JH, Leuschen WE. 1982. Controlling volunteer corn (*Zea mays*) in soybeans (*Glycine max*) with diclofop and glyphosate. *Weed Science* 30: 132-136.

Baumann PA, Dotray PA, Prostko EP. 2008. Herbicides: how they work and the symptoms they cause. Available at https://agrilifeextension.tamu.edu/library/gardening/herbicides-how-they-work-and-the-symptoms-they-cause/ [accessed 10 September 2019].

Carbonari CA, Latorre DO, Gomes GLGC, Velini ED, Owens DK, Pan Z, Dayan FE. 2016. Resistance to glufosinate is proportional to phosphinothricin acetyltransferase expression and activity in LibertyLink and WideStrike cotton. *Planta* 243: 925-933.

Chahal PS, Jhala AJ. 2015. Herbicide programs for control of glyphosate-resistant volunteer corn in glufosinate-resistant soybean. *Weed Technology* 29: 431-443.

Chahal PS, Jha P, Jackson-Ziems T, Wright R, Jhala AJ. 2016. Glyphosate-resistant volunteer maize (*Zea mays L.*): Impact and management. In:Travlos IS, Bilalis D, Chachalis D (eds), *Weed and Pest Control.* Lincoln: Nova Science Publishers. pp. 83-94.

Chahal PS, Kruger G, Blanco-Canqui H, Jhala AJ. 2014. Efficacy of pre-emergence and post-emergence soybean herbicides for control of glufosinate-, glyphosate-,and imidazolinone-resistant volunteer corn. *Journal of Agricultural Science* 6: 131-140.

Curran WS, McGlamery MD, Liebl RA, Lingenfelter DD. 1999. Adjuvants enhancing herbicide performance. *Agronomy Facts* 37: 1-12.

Czarnota M, Thomas P. 2010. Using surfactants, wetting agents and adjuvants in the greenhouse. *The University of Georgia Cooperative Extension* 1314: 1-8.

Deen W, Hamill A, Shropshire C, Soltani N, Sikkema PH. 2006. Control of volunteer glyphosate-resistant corn (*Zea mays*) in glyphosate-resistant soybean (*Glycine max*). *Weed Technology* 20: 261-266.

Duke O. 1990. Overview of herbicide mechanisms of action. *Environmental Health Perspectives* 87: 263-271.

Duke SO. 2017. The history and current status of glyphosate. *Pest Management Science* ***Number?*** *1-9.*

Funke T, Han H, Healy-Fried ML, Fischer M, Schönburn E. 2006. Molecular basis for the herbicide resistance of Roundup Ready crops. In: Matthews BW (eds), *Proceedings of the National Academy of Sciences of the United States of America.* pp. 13010-13015.

Green, J. 1992. Increasing efficiency with adjuvants and herbicide mixtures. *Proceedings of the First International Weed Control Congress, Melbourne.* pp.187-191.

Gressel J. 2010. Global advances in weed management. *Journal of Agricultural Sciences* 10: 1-7.

Harris KM, Nwanze KE. 1992. *Busseola fusca (Fuller), the African Maize Stalk Borer: a handbook of information.* Pradesh: ICRISAT.

Hazen JL. 2000. Adjuvants-terminology, classification, and chemistry. *Weed Technology* 14: 773-784.

Hilbeck A, Schmidt JEU. 2006. Another view on Bt proteins- how specific are they and what else might they do?. *Biopescticides International* 2: 1-50.

Jeschke M, Doerge T. 2008. Managing volunteer corn in cornfields. *Crop Insights* 18: 1-4.

Jordan T, Johnson B, Nice G. 2011. Adjuvants used with herbicides: factors to consider. *Purdue Extension Weed Science.* Available at HYPERLINK "https://ag.purdue.edu/btny/weedscience/Pages/default.aspx" https://ag.purdue.edu/btny/weedscience/Pages/default.aspx [accessed 17 September 2019].

Krupke C, Marquardt P, Johnson W, Weller S, Conley SP. 2009. Volunteer corn presents new challenges for insect resistance management. *Agronomy Journal* 101: 797-799.

Kumaratilake AR, Preston C. 2005. Low temperature reduces glufosinate activity and translocation in wild radish (*Raphanus raphanistrum*). *Weed Science* 53: 10-16.

Lan Y, Hoffman WC, Fritz BK, Martin DE, Lopez JD. 2008. Spray drift mitigation with spray mix adjuvants. *Applied Engineering in Agriculture* 24: 5-10.

Marquardt PT, Johnson WG. 2013. Influence of clethodim application timing on control of volunteer corn in soybean. *Weed Technology* 27: 645-648.

Marquardt PT, Terry RM, Johnson WG. 2013. The impact of volunteer corn on crop yields and insect resistance management strategies. *Agronomy* 3: 488-496.

McMullan PM. 2000. Utility Adjuvants. *Weed Technology* 14: 792-797.

Meisel B, Korsman J, Kloppers FJ, Berger DK. 2009. *Cercospera zeina* is the causal agent of grey leaf spot disease of maize in southern Africa. *European Journal of Plant Pathology* 124: 577-583.

Mucheri T. 2016. The efficacy of glufosinate-ammonium on ryegrass as influenced by different plant growth stages and different temperatures.MSc thesis, Stellenbosch University, South Africa.

Owen M. 2008. Weed species shifts in glyphosate-resistant crops. *Pest Management Science* 64: 377-387.

Owen MDK, Zelaya IA. 2005. Herbicide-resistant crops and weed resistance to herbicides. *Pest Management Science ,* Volume: 301-311.

Penner D. 2000. Activator adjuvants. *Weed Technology* 14: 785-791.

Pertile M, Cechin J, Zimmer V, Agostinetto D, Vargas L. 2018. Interference of volunteer corn in glyphosate resistant soybean and chemical control in different phenological stages. *Bioscience Journal* 34: 1248-1257.

Piasecki C, Rizzardi MA, Schwade DP, Tres M, Sartori J. 2017. Interference of GR volunteer corn population and origin on soybean grain yield losses. *Planta Daninha* 36: 1-9.

Rao V. 2000. *Herbicide interactions with herbicides, safeners and other agrochemicals (*2nd edn). Santa Clara: Science Publishers Inc.

Retzinger EJ, Mallory-Smith C. 1997. Classification of herbicides by site of action for weed resistance management strategies. *Weed Technology* 11: 384-393.

Sbatella GM, Kniss AR, Omondi EC, Wilson RG. 2016. Volunteer corn (*Zea mays*) interference in dry edible bean (*Phaseolus vulgaris). Weed Technology* 30: 937-942.

Travlos I, Cheimona N, Bilalis D. 2017. Glyphosate efficacy of different salt formulations and adjuvant additives on various weeds. *Agronomy* 7: 1-9.

Tu M, Randall JM. 2003. Adjuvants. In: Tu M, Hurd C, Randall JM (eds), *Weed Control Methods Handbook.* Virginia: The Nature Conservancy.

Xu L, Zhu H, Ozkan HE, Bagley WE, Krause CR. 2010. Droplet evaporation and spread on waxy and hairy leaves associated with type and concentration of adjuvants. *Pest Management Science* 67: 842-851.

Yuan JS, Tranel PJ, Neal Stewart C. 2006. Non-target-site herbicide resistance: a family business. *TRENDS in Plant Science* 12: 6-13.

Zollinger R. 2012. Spray adjuvants: the rest of the story. In: *Proceedings of the 24th Annual Integrated Crop Management Conference, 2*8th November. pp 81-87.

CHAPTER 3: MATERIALS AND METHODS

3.1 Trial sites

This study was conducted at two trial sites and replicated in an identical manner at both trial sites to investigate the impact of different climates on the efficacy of herbicide-adjuvant mixtures to control glyphosate-resistant volunteer maize.

3.1.1 Morgenzon

The first trial site was located outside the town of Morgenzon on the Mpumalanga highveld with coordinates 26°47’45.18”S 29°39’04.86”E (Figure 3.1).

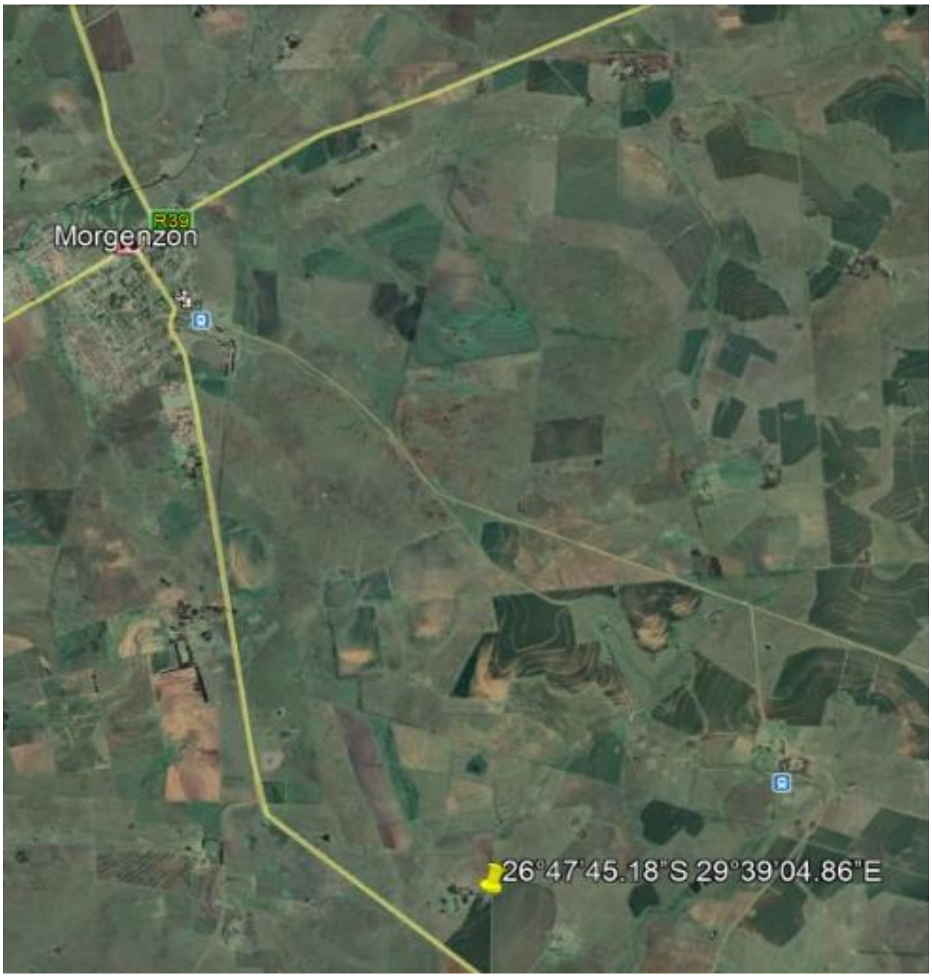


Figure 3.1: Morgenzon trial site location (Google Maps 2019a)

According to the Köppen-Geiger climate classification this area is classified as the Cwb type (Peel et al. 2007). The Cwb type is described as areas with a subtropical highland climate or a temperate oceanic climate with dry winters (Peel et al. 2007). These areas are also known for annual lower temperatures (Peel et al. 2007).

3.1.2 Nelspruit

The second trial location is situated on the outskirts of Nelspruit in the Mpumalanga lowveld with coordinates 25°26’32.91”S 30°59’33.24”E (Figure 3.2).

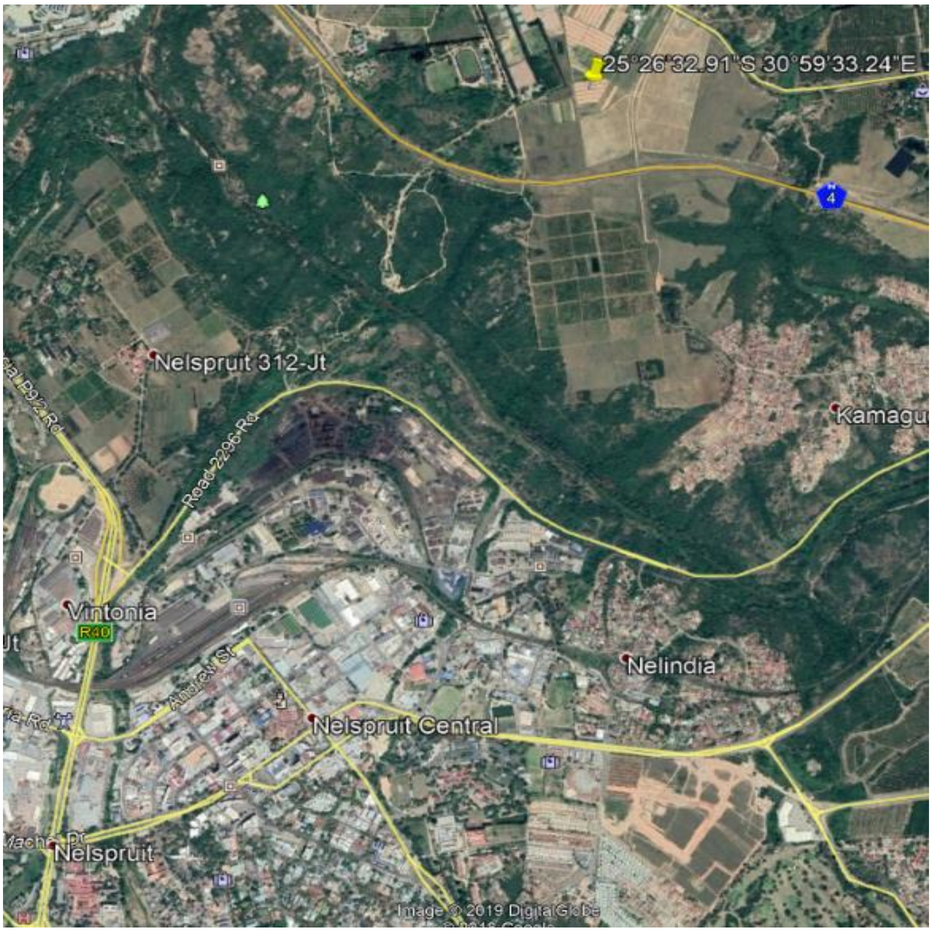


Figure 3.2: Nelspruit trial site location (Google Maps 2019b)

According to the Köppen-Geiger climate classification this area is classified as the Cwa type (Peel et al. 2007). The Cwa type is described as areas with monsoon influenced humid subtropical climates (Peel et al. 2007).

3.2 Planting

Maize seed was planted with a conventional till planter which was set to plant at a row spacing of 90 cm and inner row spacing between plants of 17 cm leading to a plant density of ± 65 000 plants per hectare. This method was preferred to broadcast sowing, which better simulates natural conditions, to achieve an even stand of maize to ensure all plots contained similar numbers of plants and the same target area applies to all the applications.

The maize was planted on dryland areas without the possibility of irrigation. This method was followed because most of the maize in Mpumalanga is grown under dryland conditions.

The PAN 6R-680RR variety was planted. This hybrid variety is glyphosate-resistant and is adapted to both trial site areas.

3.3 Trial design

The project was designed to investigate the efficacy of three different herbicides namely clethodim, quizalafop-P-tefuryl and and glufosinate ammonium, each in combination with several adjuvants, on glyphosate resistant volunteer maize. Each herbicide with its accompanying set of adjuvants were considered a separate study. Each study was split into two trials. Trial A set out to determine the efficacy of herbicide-adjuvant mixtures to control glyphosate-resistant volunteer maize at the water volume of 200 L ha^{-1} as prescribed by herbicide labels. Trial B set out to determine the influence of different water volumes on the deposition of herbicide-adjuvant mixtures and efficacy of the mixtures to control glyphosate-resistant volunteer maize. All three herbicides and their respective adjuvants trials consisted of a trial A and trial B. Trials A and B were conducted in the same manner for all three of the herbicides. During both of the trials the herbicides used were applied at half the dosage rate prescribed by the product labels to exaggerate the adjuvant influence on the efficacy of the herbicides. Due to the difference in objectives between the two trials the trial designs will be discussed separately. Trial A will be referred to as the efficacy trial and trial B will be referred to as the deposition trial.

3.3.1 Efficacy

The efficacy trial design employed a randomized complete block design (RCBD) and each treatment was replicated four times. Treatment one of each trial served as the untreated control (UTC) and is marked in red in Figures 3.3, 3.4 and 3.5. The randomization for each herbicide differed because each herbicide contained a different amount of adjuvant combinations. The clethodim study contained thirteen treatments and when the RCBD was employed the trial was demarcated as shown in Figure 3.3. The quizalofop-P-tefuryl study contained fourteen treatments and when the RCBD was employed the trial was demarcated as shown in Figure 3.4. The glufosinate-ammonium study contained twelve treatments and when the RCBD was employed the trial was demarcated as shown in Figure 3.5. In each of the three figures the treatments in the first block (A) is numbered consecutively to show the number of treatments but in the field the treatments in Block A were also randomized similar to the other three blocks. A plot width of 2 m and a plot length of 10 m was used in the efficacy trials. Each plot covered an area of 20 m^2 and was sprayed lengthwise starting at meter 0 and ending at meter 10.

A	1	2	3	4	5	6	7	8	9	10	11	12	13
B	6	9	13	10	8	4	12	1	3	11	7	5	2
C	10	7	11	1	3	13	5	12	6	2	9	4	8
D	12	8	5	9	11	2	4	7	13	1	6	10	3

Figure 3.3: Clethodim efficacy trial layout.

A	1	2	3	4	5	6	7	8	9	10	11	12	13	14
B	5	12	8	11	2	14	10	3	13	4	6	9	7	1
C	10	6	13	1	11	4	9	7	12	14	3	8	5	2
D	9	7	14	8	3	13	12	2	5	1	4	11	6	10

Figure 3.4: Quizalofop-P-tefuryl efficacy trial layout.

A	1	2	3	4	5	6	7	8	9	10	11	12
B	6	10	5	8	12	7	2	11	1	9	4	3
C	7	4	9	11	1	10	5	3	12	6	8	2
D	11	8	12	3	9	2	10	6	4	7	1	5

Figure 3.5: Glufosinate-ammoniun efficacy trial layout.

3.3.2 Deposition

The deposition trial contained the same treatment combinations as in the efficacy trial, but four different water volumes were applied to evaluate the influence of water volume on deposition and efficacy of herbicide adjuvant mixtures. Treatments were applied at 100 L ha^{-1}, 150 L ha^{-1}, 200 L ha^{-1} and 300 L ha^{-1}. To still produce four replications, per treatment, per water volume the $20\,m^2$ plots were divided into four sub-plots. Therefore one $20\,m^2$ plot, when divided looked as shown in Figure 3.6:

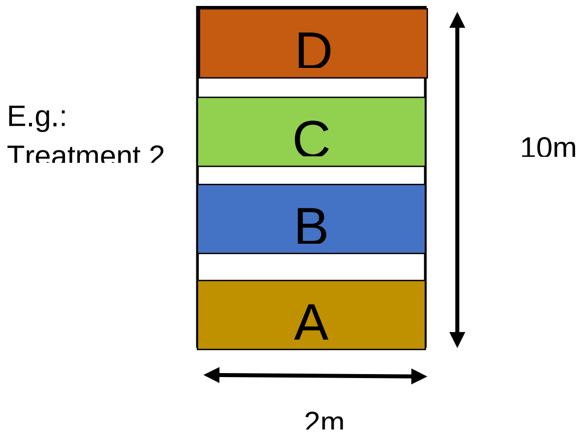


Figure 3.6: Plot design for the deposition trial.

Each subplot had measurements of 2 m x 2 m and received different water volumes. In this example Treatment 2 would have been applied at 100 L ha^{-1} in subplot A, at 150 L ha^{-1} in subplot B, at 200 L ha^{-1} in subplot C and at 300 L ha^{-1} in subplot D. The Treatment 2 plot, for example, was then replicated in the four blocks similar to the efficacy trial (See Figures 3.3 to 3.5). The subplots were then evaluated as plots and the data obtained was used to produce the deposition trial results.

By dividing the plots into subplots, the space and product availability constraints were overcome and the deposition trial could be executed.

3.4 Application

The applications for the efficacy and deposition trials were done by using the same CO_2 boom sprayer (Figure 3.7). The sprayer has a boom length of 1.8 m with four nozzles spaced 50 cm apart. When held at a height of 50 cm above the target area the sprayer produced a spray width of 2 m. For the efficacy trial the same nozzle was used throughout the application process and the tank pressure stayed constant at 2.0 bars of pressure. The nozzle used was an XR TEEJET 11002 nozzle and with a 2 bar pressure the sprayer delivered 205 L ha^{-1}.

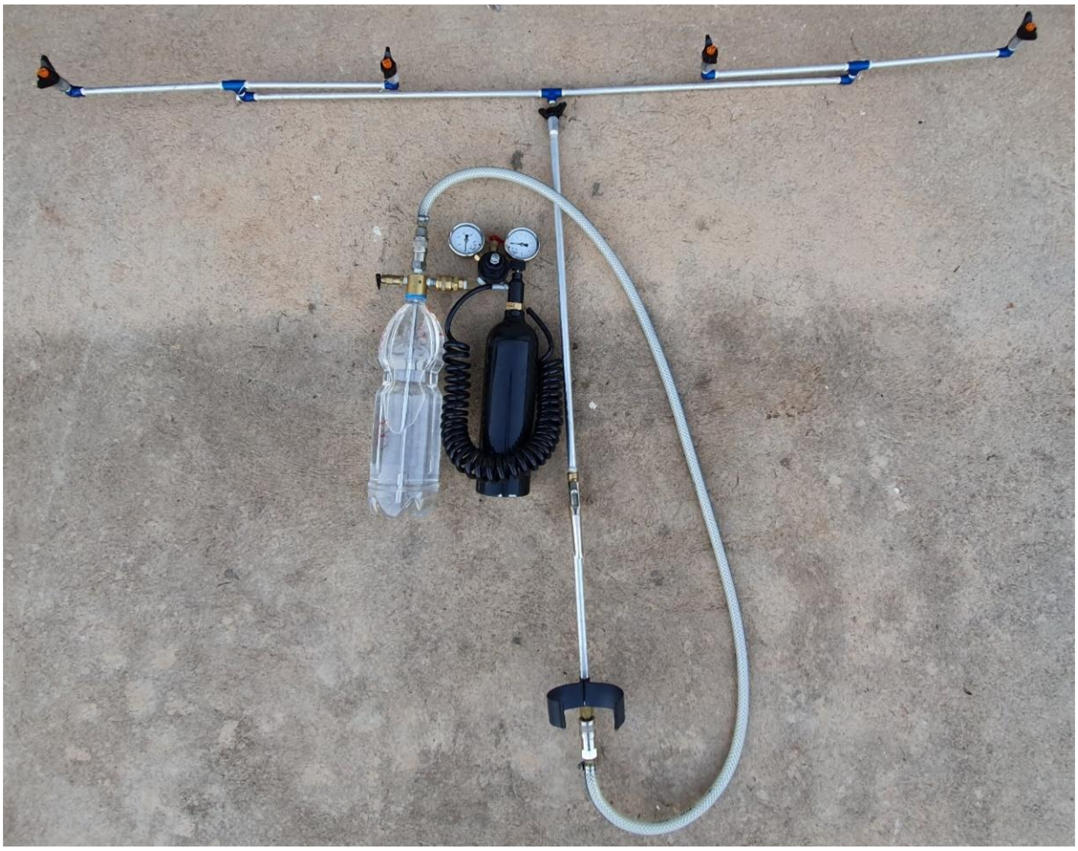


Figure 3.7: CO_2 boom sprayer used for application of treatments.

The deposition trial was sprayed with four different nozzles to deliver the required water volumes (Figures 3.8 to 3.11) and the tank pressure was kept as constant as possible to avoid unnecessary drift or droplet size variations (Table 3.1). The selection of different nozzles will also deliver different droplet spectrums. Therefore, the droplet spectrum was not taken into account when measuring deposition. The deposition was solely determined by measuring the percentage coverage irrelevant of droplet spectrum.

Table 3.1*:* Nozzles and pressures used to deliver water volume during the deposition trial

Desired water volume	Nozzle used	Tank pressure
100L ha^{-1}	XR TEEJET 11001 (Figure 3.8)	2.0 bar
150L ha^{-1}	XR TEEJET 110015 (Figure 3.9)	2.0 bar
200L ha^{-1}	XR TEEJET 11002 (Figure 3.10)	2.0 bar
300L ha^{-1}	XR TEEJET 11003 (Figure 3.11)	2.2 bar

Figure 3.8: XR TEEJET 11001 used to deliver water volumes of 100 L ha^{-1}.

Figure 3.9: XR TEEJET 110015 used to deliver water volumes of 150 L ha^{-1}.

Figure 3.10: XR TEEJET 11002 used to deliver water volumes of 200 L ha^{-1}.

Figure 3.11: XR TEEJET 11003 used to deliver water volumes of 300 L ha^{-1}.

Treatments were applied once at both the trial sites when the maize plants were at the growth stages where four-and five leaves were completely unfolded. The application at the Morgenzon trial site took place on the 12th of December 2018 and on the 9th of January 2019 in Nelspruit.

3.5 Parameters assessed

3.5.1 Efficacy trial parameters

During the efficacy trial two main parameters have been assessed. The first was mortality which is expressed as a percentage. The evaluation was done 28 days after application (DAA). Twenty randomly selected plants were evaluated per plot and rated as dead or alive. For quizalofop-P-tefuryl and clethodim the whorl of the maize plant was pulled upwards very gently. If the whorl detached from the growth point and was able to be removed the plant was classified as dead (Figure 3.12). The glufosinate-ammonium plants were declared as dead when there were no visible signs of green leaf tissue (Figure 3.13).

The second parameter evaluated was the percentage necrosis and stunting caused by the herbicides. This was done 28 DAA. Necrosis is defined as the death of tissue through injury or disease (Gunther and Egel 2015). An example of necrosis is illustrated in Figure 3.14.

Stunting is defined as the slowing or lack of growth and development of a plant (Gunther and Egel 2015). An example of stunting is illustrated in Figure 3.15. The area inside the blue lines shows the growth of an untreated control plot. The area inside the green lines show the stunted growth of glyphosate resistant volunteer maize caused by herbicide application.

Figure 3.12: Whorl detachment from the growth point of glyphosate resistant volunteer maize plants treated with quizalofop-P-tefuryl and clethodim.

Figure 3.13: Absence of green leaf tissue on glyphosate resistant volunteer maize plants treated with glufosinate ammonium.

Figure **3.14:** Necrosis of glyphosate-resistant volunteer maize after herbicide treatment.

Figure 3.15: Stunting of glyphosate-resistant volunteer maize after herbicide treatment. The area inside the blue lines shows the growth of an untreated control plot. The area inside the green lines show the stunted growth of glyphosate resistant volunteer maize caused by herbicide application.

Due to the influence of climatic conditions on the efficacy of herbicides and the differences in local climate between the two trial locations, weather conditions were also measured with the focus on temperature differences. Temperature at the time of application, as well as temperatures before application and after application was obtained from data, supplied by the Agricultural Research Council (ARC). Although the focus was on temperature data other environmental factors such as precipitation, humidity and wind speed were also measured.

For the Morgenzon trial site, weather data for November 2018, December 2018 and January 2019 were supplied by the ARC. The three months covered weather data from the time of planting until the conclusion of the trial to ensure a thorough data analyses is possible.

For the trial in Nelspruit, weather data for December 2018, January 2019 and February 2019 were supplied by the ARC.

3.5.2 Deposition trial parameters

During the deposition trial the same parameters were tested as in the efficacy trial to investigate the effect of water volume on these parameters. To evaluate the impact of water volume on deposition water sensitive papers (WSP) were attached to the upper leaf surface of maize plants (Figure 3.16). The WSP turns blue when water is deposited on the surface which provided a trusted medium on which to evaluate deposition (Figure 3.17). One WSP was placed in each deposition sub-plot to ensure four replications are available for data analysis.

Figure 3.16: Water sensitive paper attached to the upper surface of a maize plant.

Figure 3.17: Water sensitive paper turning blue after water deposition.

3.6 Evaluation methods

The evaluation for mortality has already been discussed but here follows the simple equation to express mortality as a percentage: $\frac{Number\ of\ dead\ plants}{20}$ x 100. Although the evaluation method differed slightly for the different herbicides the percentage mortality equation remained constant. Necrosis and stunting were evaluated by viewing the plot and rating the percentage necrosis and stunting throughout the entire plot.

To determine the deposition differences the aim was to establish the percentage cover that an herbicide-adjuvant mixture achieved when sprayed at the different water volumes. This was done by fixing the WSP's (2.6 x 4.0 cm, Syngenta) to a A4 paper and scanned using a Konica Minolta bizhub c364e scanner resulting in a 24-bit colour image of size (614 x 944 pixels). A scanning resolution of 600 dpi was used based on a previous study (Cunha et al. 2012) which found this to be most suitable. Colour images were imported into ImageJ (Collins 2007) and converted to 8-bit grey scale images. A threshold method was applied during which the stains appear as 1 (black) and the background as 0 (white) to create a binary image. The % area was then determined for each sprayed paper.

3.7 Data analysis

One-way ANOVA analyses (Statistica version 13.5) was conducted for the efficacy trial to test for differences between adjuvant treatments. Two-way ANOVA analyses was conducted for the deposition trial to test for interactions between water volume and adjuvant treatments as well as differences within these factors. Where differences between treatments of interactions between factors were significant ($p \leq 0.05$) the means were separated by means of Fisher's LSD post hoc tests.

3.8 References

Collins T. 2007. Introduction to ImageJ for light microscopy. *Microscopy and Microanalysis* 13:1674-1675.

Cunha M, Carvalho C, Marcal A. 2012. Assessing the ability of image processing software to analyse. *Biosystems Engineering* 3: 11-23.

Google Maps. 2019a. Google Maps. Available at https://www.google.com/maps/@-26.77064,29.65609,22393m/data=!3m1!1e3 [accessed 29 April 2019].

Google Maps. 2019b. Google Maps. Available at HYPERLINK "https://www.google.com/maps/@-25.45795,30.98851,11323m/data=!3m1!1e3" https://www.google.com/maps/@-25.45795,30.98851,11323m/data=!3m1!1e3 [accessed 29 April 2019].

Gunther C, Egel D. 2015. Purdue Extension. Available at HYPERLINK "https://extension.purdue.edu/extmedia/ID/ID-319-W.pdf" https://extension.purdue.edu/extmedia/ID/ID-319-W.pdf [accessed 30 April 2019].

Peel MC, Finlayson BL, McMahon TA. 2007. Updated world map of the Köppen-Geiger climate classification. Earth System Science 11:1633-1644. Available at HYPERLINK "https://doi.org/10.5194/hess-11-633-2007" https://doi.org/10.5194/hess-11-633-2007 [accessed 17 September 2019].

Chapter 4: Quizalofop

4.1 Introduction:

Quizalofop-P-tefuryl is an ACCase inhibitor herbicide used to control glyphosate-resistant volunteer maize. To determine the influence of adjuvants on the efficacy of quizalofop-P-tefuryl to control glyphosate-resistant volunteer maize, the herbicide was combined with five different adjuvants in different combinations (Table 4.2).

The herbicide-adjuvant mixtures were then tested in a deposition and efficacy trial to determine the influence of these adjuvants on the efficacy of quizalofop-P-tefuryl to control the volunteer maize. The deposition trial consisted of necrosis, stunting, mortality and coverage data at different water volumes. The efficacy trial consisted of necrosis, stunting and mortality data at a constant water volume.

4.2 Materials and Methods:

The materials and methods as discussed in Chapter 3 is applicable to quizalofop. In this chapter the spraying protocol, which illustrates the different treatment combinations, will be presented. This protocol is the same for both the deposition and efficacy trials.

4.2.1 Protocol

As discussed in Chapter 2, five adjuvants were employed to test the impact adjuvants have on the efficacy of herbicides. In Chapter 2 the five adjuvants were identified as a surfactant/oil adjuvant combination, a surfactant/fertilizer combination, a high surfactant oil concentrate (HSOC) methylated seed oil, a liquid AMS/surfactant/humectant combination and a deposition agent. The adjuvants will be discussed by referring to the product names of these adjuvants (Table 4.1). The product Antoka 240 EC was used for the active ingredient quizalofop-p-tefuryl.

Table 4.1: Adjuvants and trade names used in this study

Adjuvant	Product name
Surfactant/oil adjuvant combination	Direct
A high surfactant oil concentrate (HSOC) methylated seed oil	Destinaire™
A surfactant/fertilizer combination	Summit Super
A liquid AMS/surfactant/humectant combination	Class Act NG™
Deposition agent	Interlock™

The treatment protocol is available in Table 4.2 and illustrates the different quizalofop-p-tefuryl and adjuvant combinations. It is worth mentioning again that the rate used for quizalofop-p-tefuryl is half the rate prescribed by the product label. This was done to exaggerate the adjuvant influence on the efficacy of the herbicides.

Table 4.2: Treatment protocol

Treatment	**Product combination**	**Dosage rate:** L ha^{-1}; v/v
1	Untreated control (UTC)	
2	Quizalofop-p-tefuryl	0.5
3	Quizalofop-p-tefuryl + Direct	0.5 + 0.1%
4	Quizalofop-p-tefuryl + Destinaire™	0.5 + 1
5	Quizalofop-p-tefuryl + Summit Super	0.5 + 0.3%
6	Quizalofop-p-tefuryl + Class Act NG™	0.5 + 2%
7	Quizalofop-p-tefuryl + Interlock™	0.5 + 0.3
8	Quizalofop-p-tefuryl + Direct® + Class Act NG™	0.5 + 0.1% + 2%
9	Quizalofop-p-tefuryl + Destinaire™ + Class Act NG™	0.5 + 1 + 2%
10	Quizalofop-p-tefuryl + Summit Super + Class Act NG™	0.5 + 0.3% + 2%
11	Quizalofop-p-tefuryl + Direct® + Interlock™	0.5 + 0.1% + 0.3
12	Quizalofop-p-tefuryl + Destinaire™ + Interlock™	0.5 + 1 + 0.3
13	Quizalofop-p-tefuryl + Summit Super + Interlock™	0.5 + 0.3% + 0.3
14	Quizalofop-p-tefuryl + Class Act NG™ + Interlock™	0.5 + 2% + 0.3

The results for quizalofop-p-tefuryl will be discussed under the following headings: Deposition and efficacy. The deposition results are based on the data retrieved from applying the treatments at 100, 150, 200 and 300 L water ha^{-1}. Coverage data for the deposition trial was obtained from analyzing the water sensitive paper that was placed in the deposition plots with the purpose of examining the influence that water volume and adjuvants have on the coverage of herbicides. The efficacy results are based on the efficacy trial done where the treatments were applied at 200 L water ha^{-1}.

The deposition and efficacy will be discussed separately for each trial site. The Morgenzon trial site will be referred to as Trial site 1 and the Nelspruit trial site will be referred to as Trial site 2.

4.3 Results

4.3.1 Morgenzon

The trial site at Morgenzon was a deposition and efficacy trial.

4.3.1.1 Deposition

The necrosis, stunting and mortality data from 28 days after application (DAA) was used to investigate the impact of different water volumes on the efficacy of herbicide-adjuvant mixtures.

Necrosis, stunting, mortality and coverage results will be presented and discussed separately.

4.3.1.1.1 Necrosis

The data from 28 DAA caused that there was significant interaction ($p<0.05$), between treatments and water volumes. A clear trend was noticed that higher necrosis was observed as the water volume increased (Figure 4.1). Furthermore, it is quite evident that necrosis increases with the addition of an adjuvant with the exception of Direct (Treatment 3) being applied solo with quizalofop-p-tefuryl (Figure 4.1).

The addition of Destinaire™ solo (Treatment 4) at any volume proved to increase the necrosis severity when compared to the remaining four adjuvants when applied solo with quizalofop-p-tefuryl except for Summit Super and Class Act NG™ at 300 L ha^{-1} (Treatments 5 and 6 respectively) (Figure 4.1). A combination of adjuvants, along with quizalofop-p-tefuryl, did not provide convincing evidence to conclude that more than one adjuvant in a tank mix will increase volunteer maize necrosis (Figure 1). The analysis of time taken to reach 80% necrosis revealed that treatments reached this threshold quicker at a water volume of 100 L ha^{-1} (Figure 4.2). This is due to the fact that very low necrosis percentages were achieved when treatments were applied at 100 L ha^{-1} (Figure 4.1). Because of this the 80% threshold was reached in a shorter time period but the necrosis did not increase over time. Figure 4.2 shows readings of 0 days for treatments 2 and 3 at 100 L ha^{-1}. This is due to the absence of necrosis at 100 L ha^{-1}.

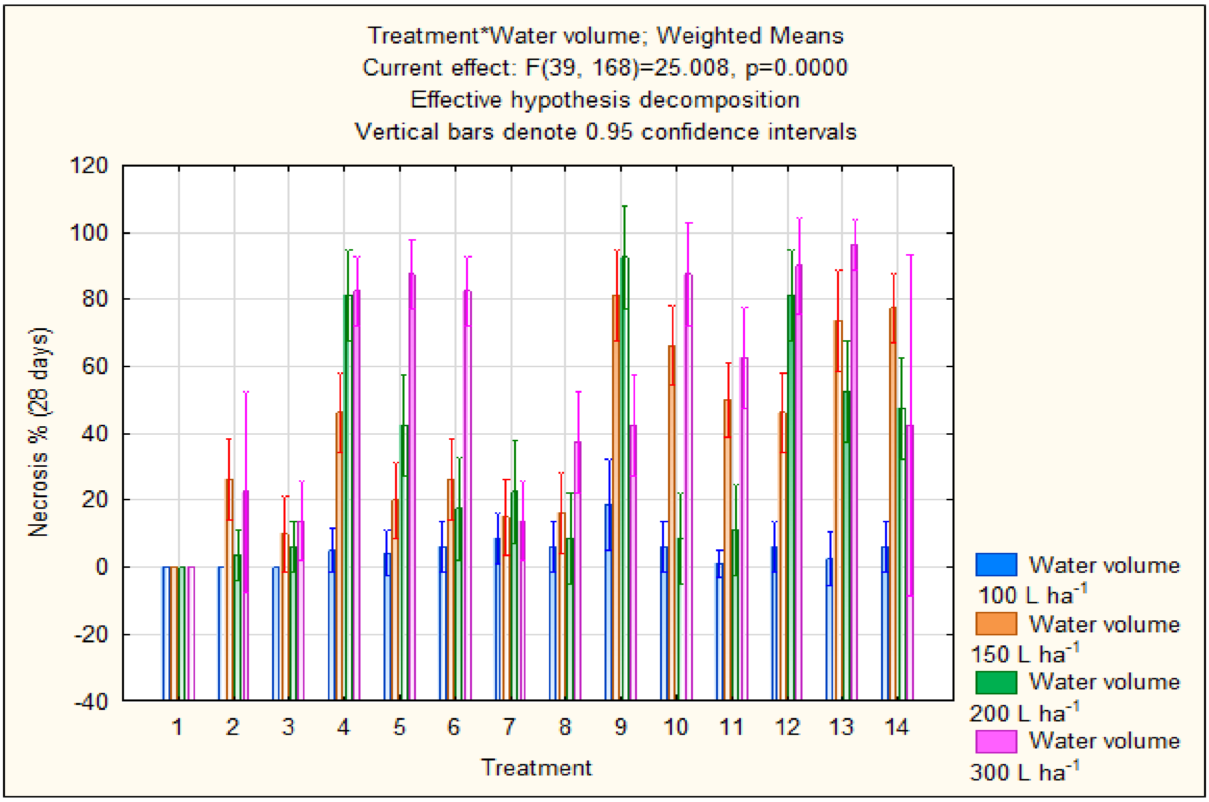


Figure 4.1: Effects of quizalofop-p-tefuryl in combination with various adjuvants on necrosis at 28 DAA, Morgenzon. (See Table 4.2 for treatment descriptions).

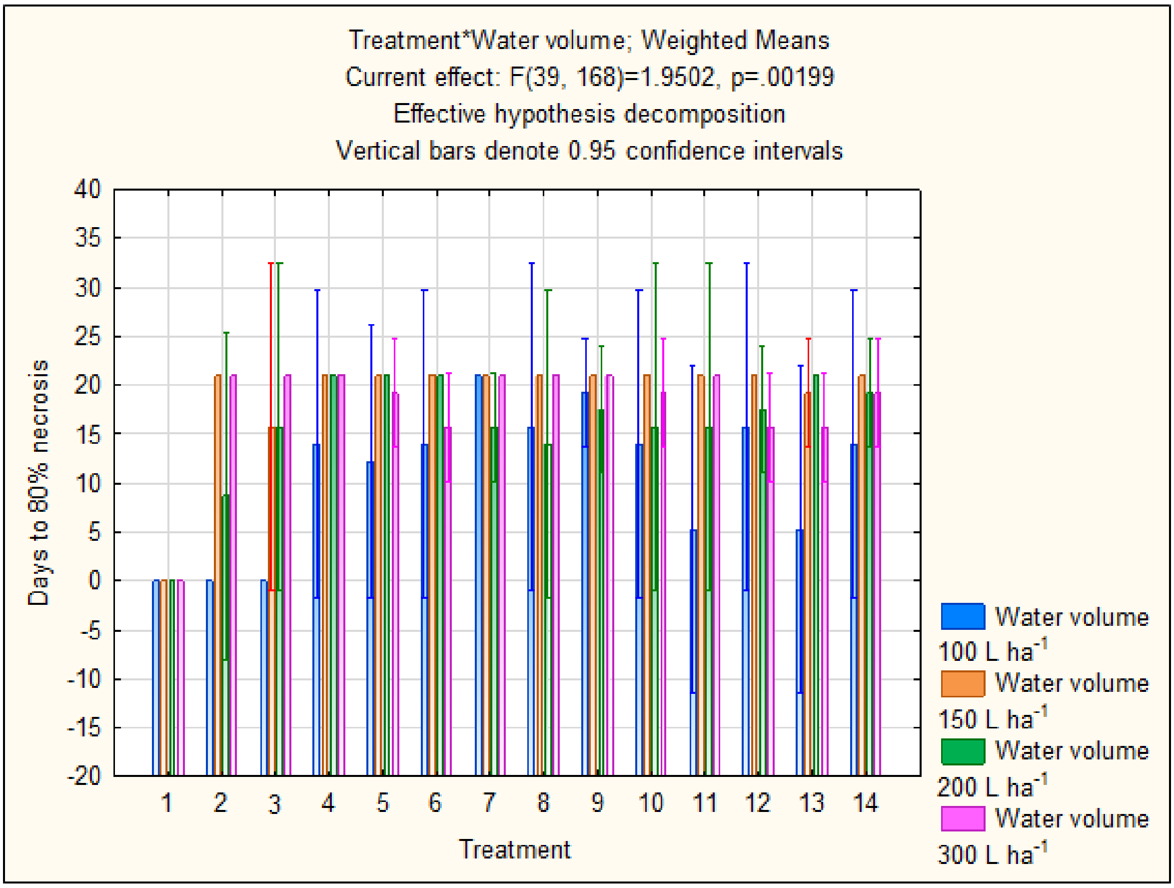


Figure 4.2: The effects of different adjuvants added to quizalofop-p-tefuryl on days to 80% necrosis threshold at 28 DAA, Morgenzon. (See Table 4.2 for treatment descriptions).

4.3.1.1.2 Stunting

The stunting analysis of quizalofop-p-tefuryl at 28 DAA revealed a very similar picture to the necrosis at 28 DAA. When the water volume increased the stunting percentage also increased (Figure 4.3). When the relationship between treatments and water volumes is investigated it is clear that the addition of Destinaire™ (Treatment 4) aids in stunting volunteer maize (Figure 4.4). When applied solo, with quizalofop-p-tefuryl, Destinaire™ caused higher stunting percentages throughout the water volume spectrum when compared with the remaining solo adjuvants and quizalofop-p-tefuryl mixtures. Using more than one adjuvant did not yield a noteworthy increase in stunting (Figure 4.4).

An increase in water volume slowed the time taken to reach the 80% stunting threshold (Figure 4.5). This may be due to a lack of stunting at the lower water volumes (Figure 4.4).

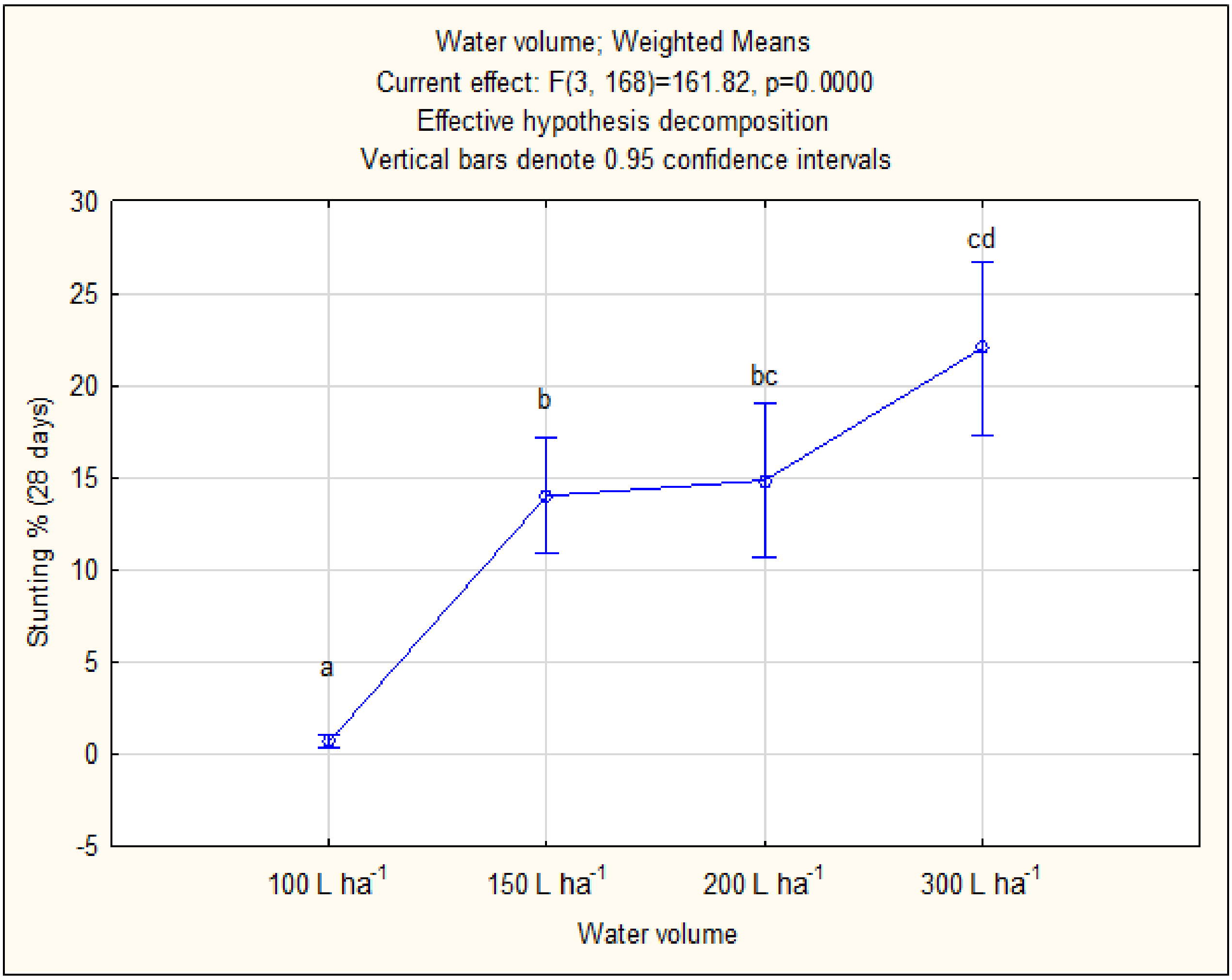


Figure 4.3: The effect of water volume on stunting of volunteer maize at 28 DAA, Morgenzon. Different letters at data points indicate significant differences between treatments at p = 0.05.

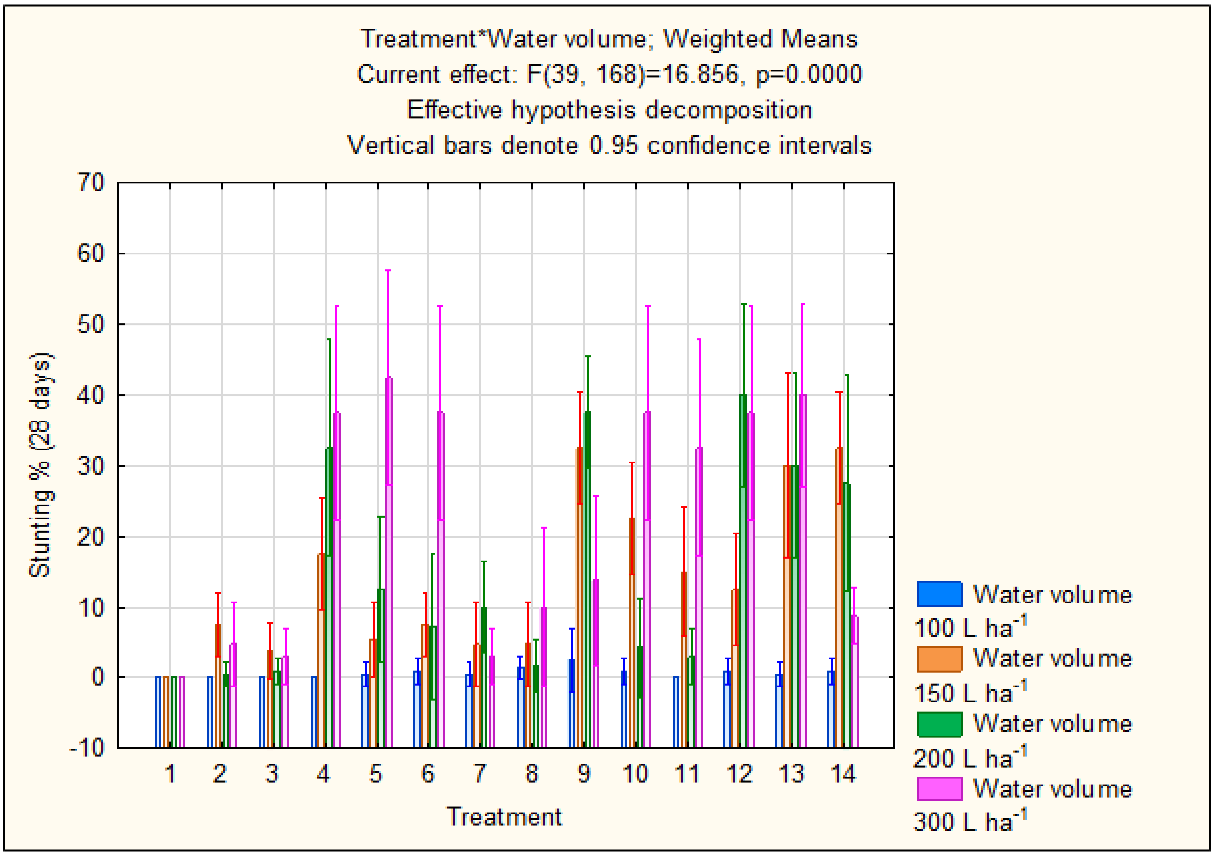


Figure 4.4: Effects of quizalofop-p-tefuryl in combination with various adjuvants on stunting at 28 DAA, Morgenzon. (See Table 4.2 for treatment descriptions).

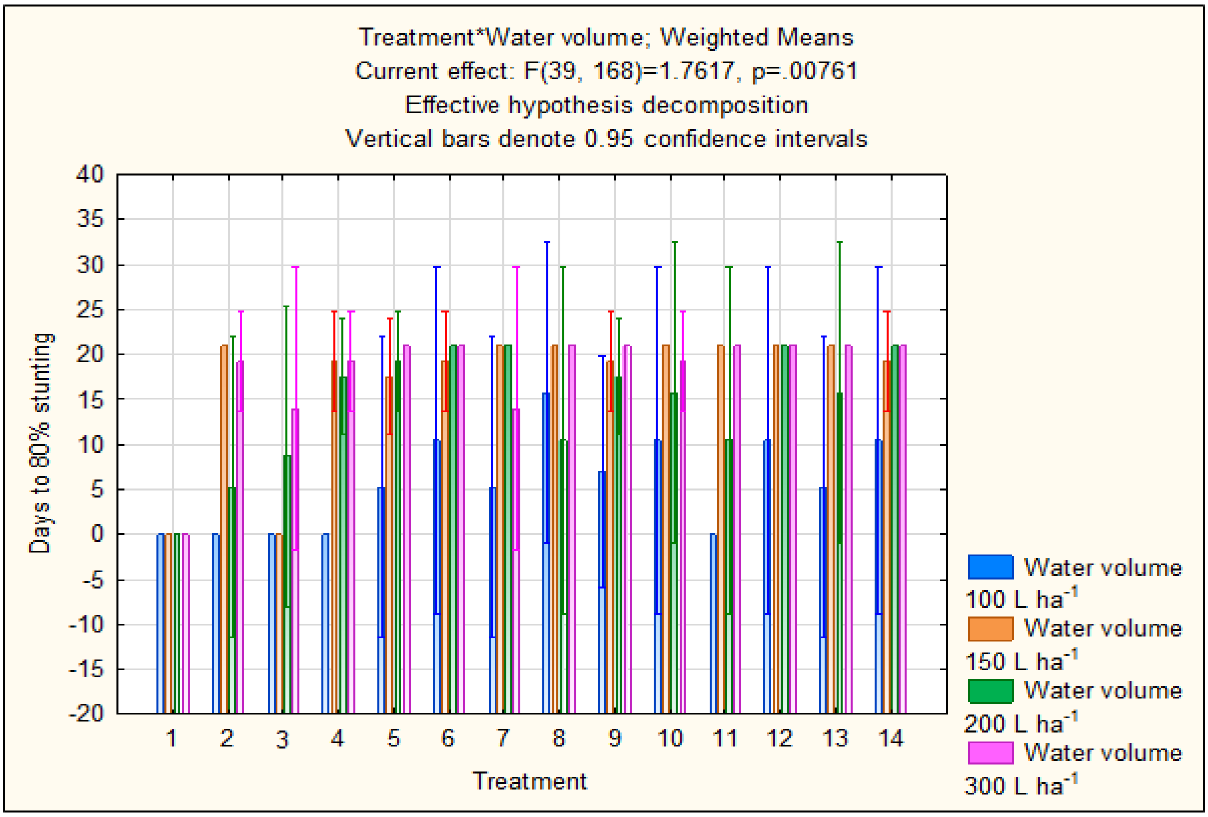


Figure 4.5 The effects of different adjuvants added to quizalofop-p-tefuryl on days to 80% stunting threshold at 28 DAA, Morgenzon. (See Table 4.2 for treatment descriptions).

4.3.1.1.3 Mortality

The mortality rating at 28 DAA revealed that there are significant interactions ($p<0.05$) between treatments and water volumes in terms of mortality. Previous trends of necrosis and stunting increasing with water volume increases are evident when mortality is analyzed (Figure 4.6).

When applied at 300 L ha^{-1} the mortality rate was significantly higher. That trend is clearer when mortality is observed compared to necrosis and stunting. During the necrosis and stunting analysis, the general trend was that a higher water volume leads to higher percentages of volunteer maize injury but there were peaks and dips at irregular intervals. The mortality analysis provides a more constant upwards curve which reveals that even though the plant did not appear to be injured the whorl did detach and the plant was classified as dead (Figure 4.6).

Figure 4.6 further shows that Destinaire™ (Treatment 4) was once again the adjuvant that proved most successful when applied solo with quizalofop-p-tefuryl. At 150, 200 and 300 L ha^{-1} Destinaire™ achieved higher mortality rates than the remaining solo adjuvant mixtures with quizalofop-p-tefuryl except for Summit Super and Class Act NG™ (Treatments 5 and 6 respectively) at 300 L ha^{-1} (Figure 4.6). In combination with Class Act NG™, Destinaire™ provided the highest mortality rates at 100 150 and 200 L ha^{-1} respectively when applied with quizalofop-p-tefuryl. When combined with Interlock™ (Treatment 7) and quizalofop-p-tefuryl, Destinaire™ also provided significantly higher percentages of mortality compared to the other treatments (Figure 4.6). The days to 80% mortality analysis revealed that when applied at higher water volumes the threshold takes longer to reach (Figure 4.7). This may be due to a lack in high mortality percentages at lower water volumes (Figure 4.6).

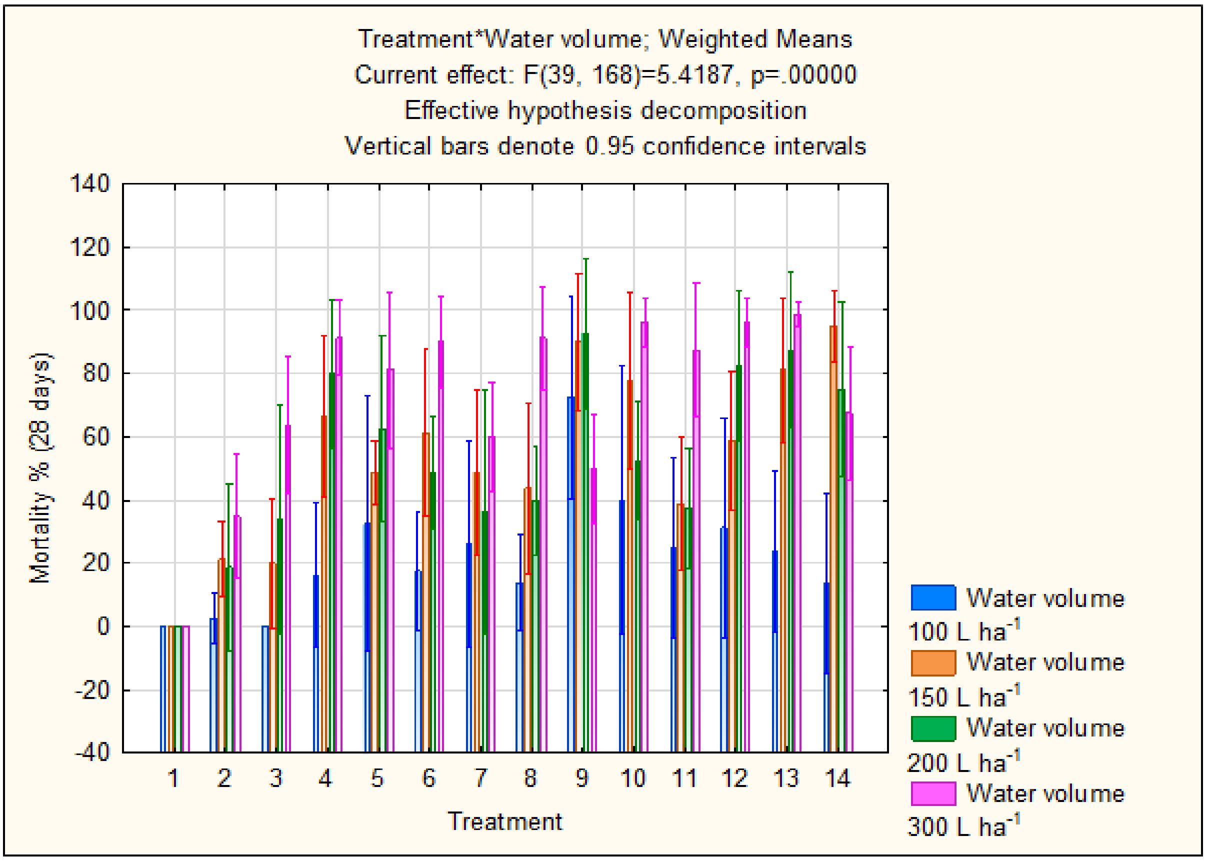


Figure 4.6: Effects of quizalofop-p-tefuryl in combination with various adjuvants on percentage mortality of volunteer maize at 28 DAA, Morgenzon. (See Table 4.2 for treatment descriptions).

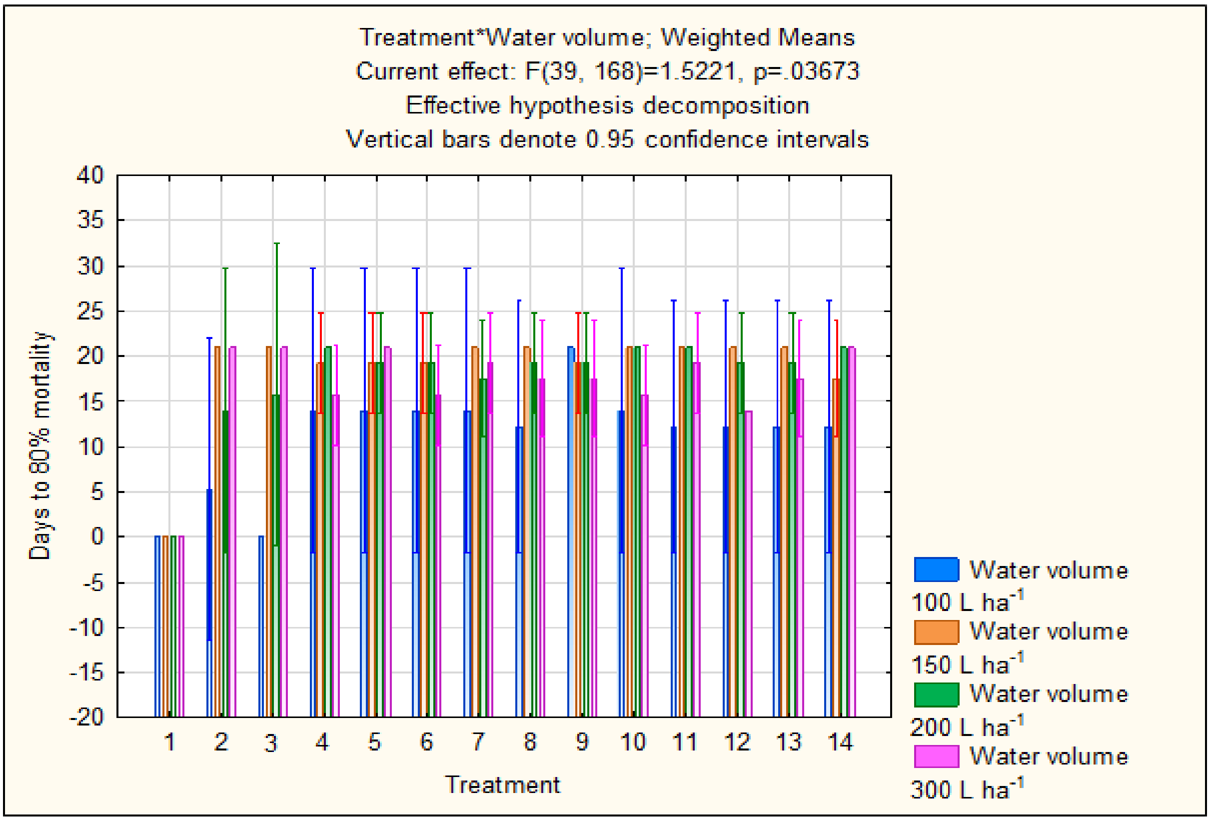


Figure 4.7: The effects of different adjuvants added to quizalofop-p-tefuryl on days to 80% mortality threshold at 28 DAA, Morgenzon. (See Table 4.2 for treatment descriptions).

4.3.1.1.4 Coverage

A significant interaction was observed between water volumes and treatments ($p<0.05$). An increase in water volume led to an increase in coverage (Figure 4.8). Class Act NG™ (Treatment 6) combined with quizalofop-p-tefuryl delivered the highest percentage coverage at 300 L ha^{-1} (Figure 4.8). Quizalofop-p-tefuryl combined with Direct (Treatment 3) and Class Act NG™ delivered a high coverage percentage at both 100 L ha^{-1}as well as 200 L ha^{-1}. Destinaire™ (Treatment 4) tank mixed with Class Act NG™ and quizalofop-p-tefuryl provided high percentage coverage at 200 L ha^{-1}compared to the other treatments at 200 L ha^{-1} (Figure 4.8). Class Act NG™ features in the treatments that delivered substantial coverage.

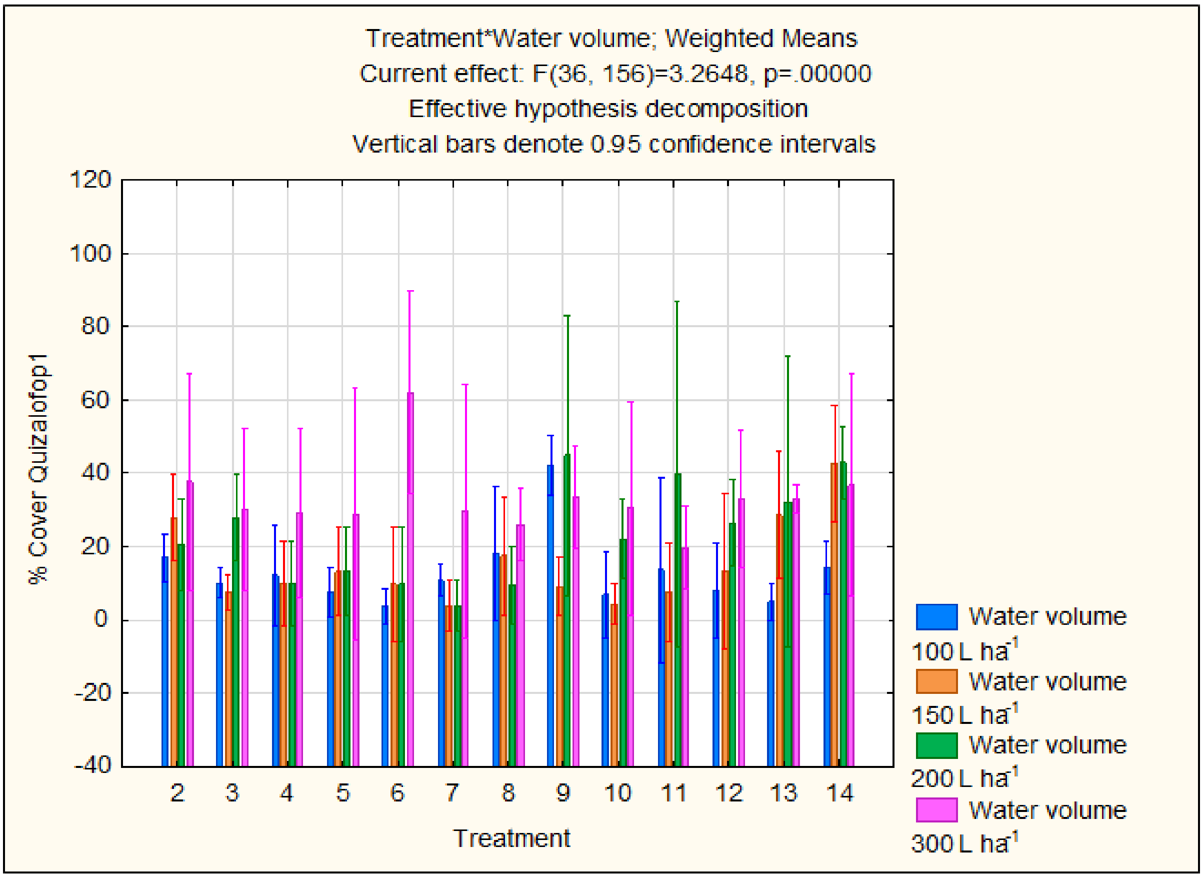


Figure 4.8: Effects of quizalofop-p-tefuryl in combination with various adjuvants on percentage coverage of volunteer maize at 28 DAA, Morgenzon. (See Table 4.2 for treatment descriptions).

Also, worth mentioning is that the deposition agent, Interlock™ (Treatment 7), did not aid in the coverage of the herbicide-adjuvant mixtures as was expected. Interlock™ did not improve coverage as a solo adjuvant, nor in a combination with an additional adjuvant.

The treatment and water volume interaction did highlight certain products but an inconsistency by all the product combinations did occur therefore the conclusion may be made that water volume, rather than treatments, influences the coverage of herbicides as is evident in Figure 4.9.

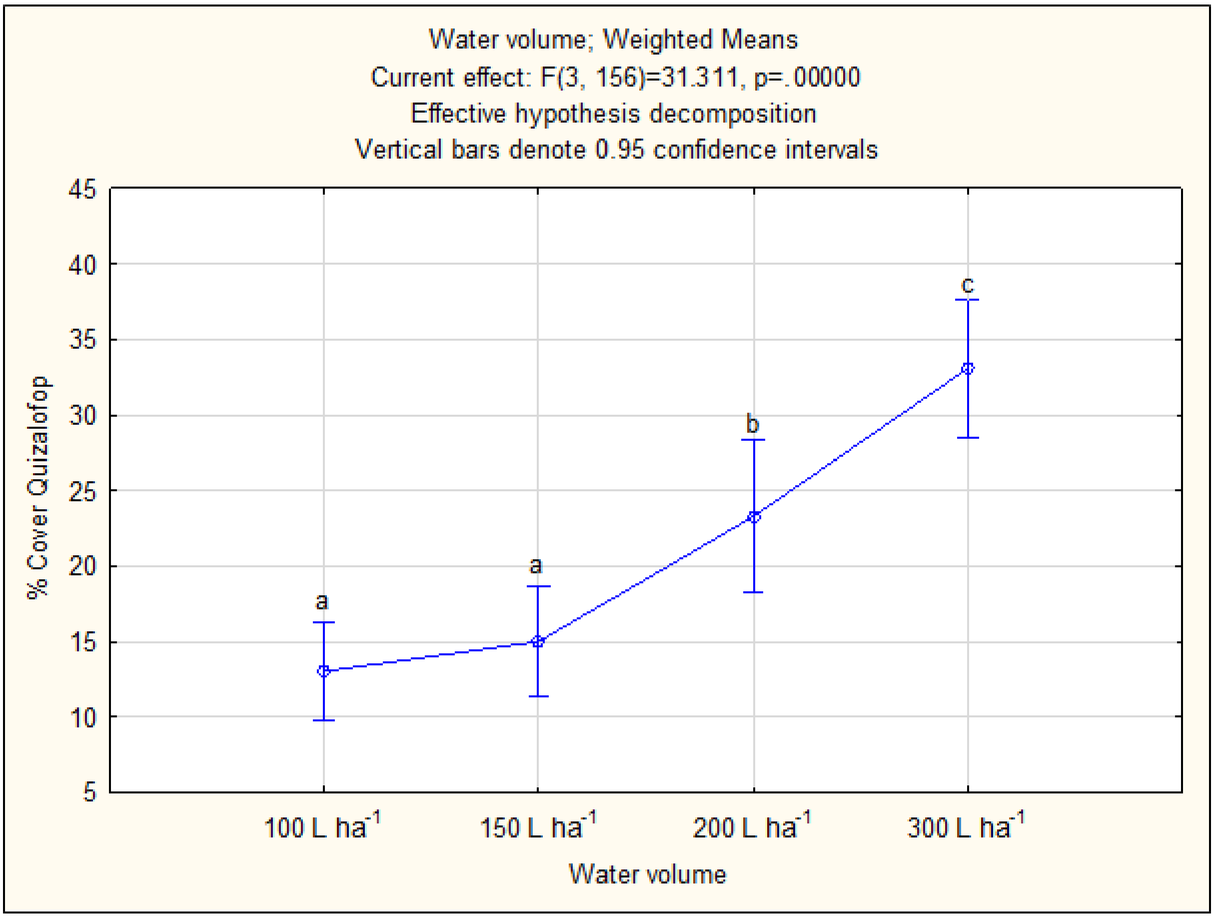


Figure 4.9: The effect of water volume on coverage of the herbicide on volunteer maize plants, Morgenzon. Different letters at data points indicate significant differences between treatments at p = 0.05.

4.3.1.2 Efficacy

The necrosis, stunting and mortality data at 28 DAA was used to determine the efficacy of herbicide-adjuvant mixtures to control glyphosate-resistant volunteer maize.

4.3.1.2.1 Necrosis

The data obtained revealed that there were significant differences between the treatments ($p<0.05$). A combination of Summit Super + Interlock™ (Treatment 13) caused a significantly higher necrosis percentage compared to all the solo adjuvant applications as well as adjuvant combinations treatments 8, 10, 11 and 14 (Figure 4.10). No differences were observed between the solo adjuvant applications, but Destinaire™ + quizalofop-p-tefuryl did provide numerically the highest necrosis percentage at 13.75% (Figure 4.10). The treatments that caused the highest necrosis percentages, all contained Summit Super, Class

Act™, Destinaire™ and Interlock™. Destinaire™ features most prominently and is present in treatments 4, 9 and 12.

4.3.1.2.2. Stunting

Significant differences were observed between treatments ($p<0.05$) when stunting is analysed. A combination of Summit Super + Interlock™ (Treatment 13) provided the highest stunting percentage at 11.25%. This was significantly higher than Treatments 1,2,3,5,6,7,8,10,11 and 14 (Figure 4.11). Treatment 4, Destinaire™ + quizalofop-p-tefuryl, delivered the highest numerical stunting percentage at 5.5%, for a solo adjuvant treatment, which does not differ statistically from Treatment 13 which provided the highest stunting percentage (Figure 4.11).

4.3.1.2.3. Mortality

Treatments 4, 9,12 and 13 provided the highest percentage mortality but these treatments did not differ from one another (Figure 4.12). Treatment 4 is the only solo adjuvant mixture that provided a relatively high mortality percentage and consisted of Destinaire™ and quizalofop-p-tefuryl. Destinaire™ also features in treatments 9 and 12 which is two of the treatments that produced the highest mortality percentage (Figure 4.12).

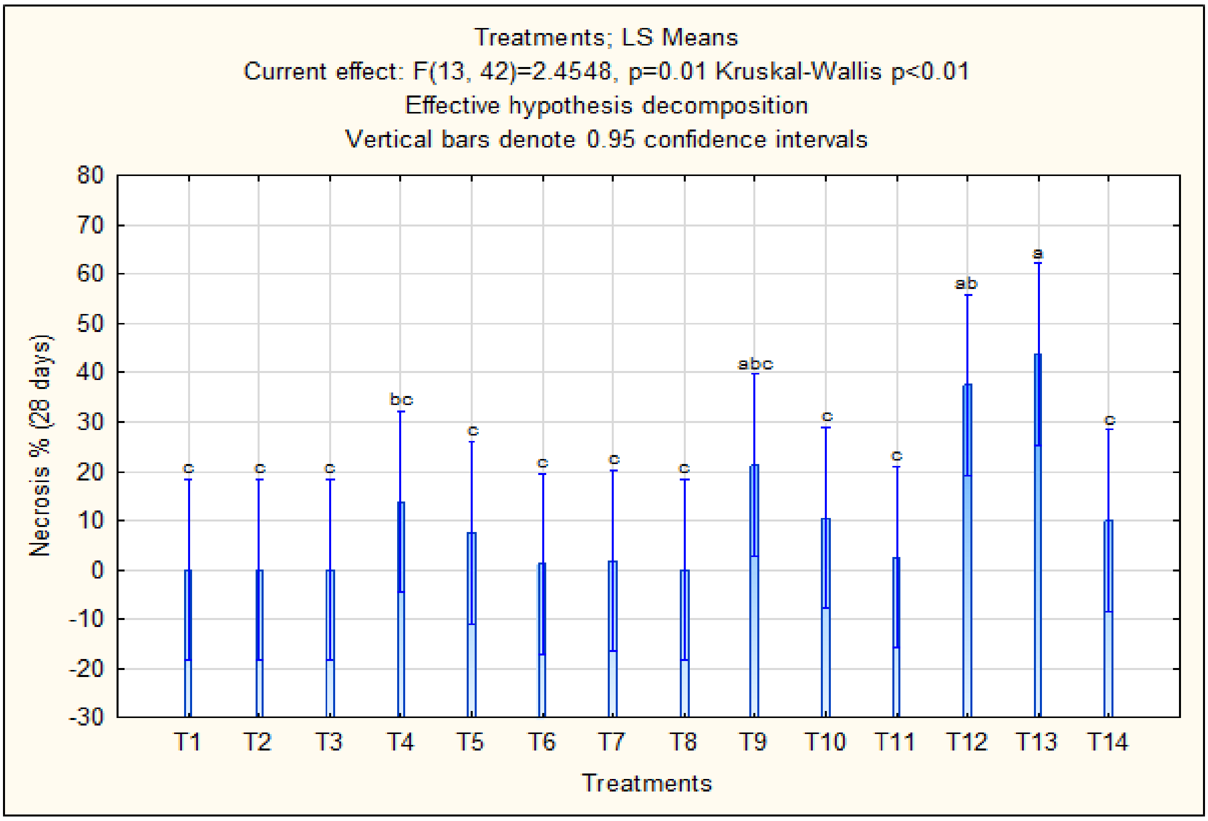


Figure 4.10: Effects of quizalofop-p-tefuryl in combination with various adjuvants on the efficacy to cause necrosis at 28 DAA, Morgenzon. (See Table 4.2 for treatment descriptions). Different letters at data points indicate significant differences between treatments at p = 0.05.

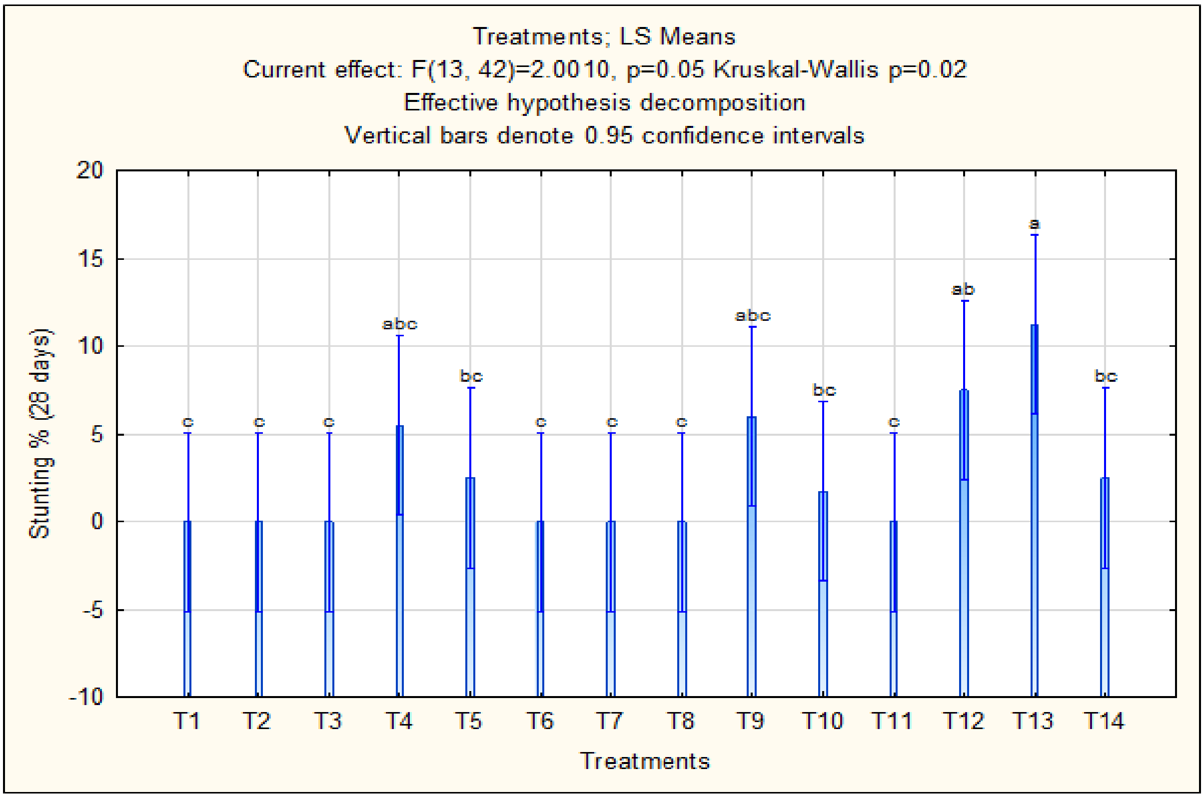


Figure 4.11: Effects of quizalofop-p-tefuryl in combination with various adjuvants on the efficacy to cause stunting at 28 DAA, Morgenzon. (See Table 4.2 for treatment descriptions). Different letters at data points indicate significant differences between treatments at p = 0.05.

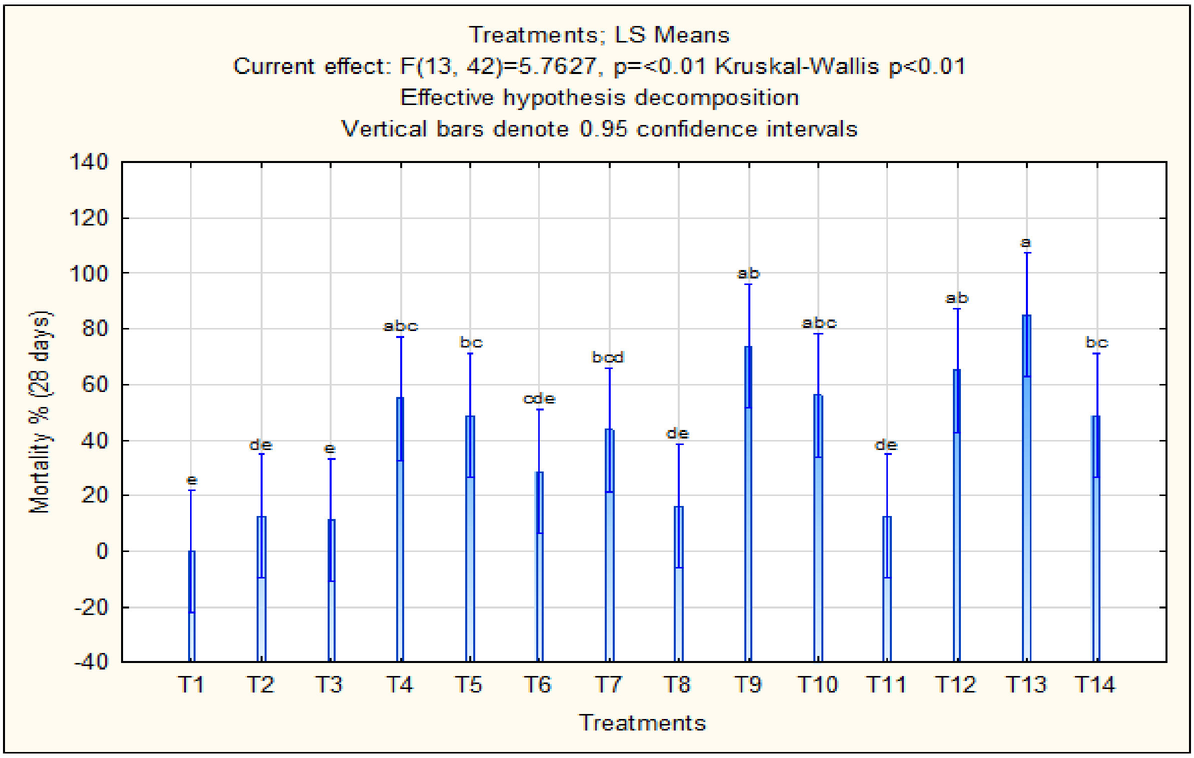


Figure 4.12: Effects of quizalofop-p-tefuryl in combination with various adjuvants on the efficacy to cause mortality at 28 DAA, Morgenzon. (See Table 4.2 for treatment descriptions). Different letters at data points indicate significant differences between treatments at p = 0.05.

4.3.2 Nelspruit

The trial site at Nelspruit was subjected to a deposition and efficacy trial and the results from that trial site will be caused here.

4.3.2.1 Deposition

The purpose and methods used to evaluate the deposition trial in Morgenzon was the same for Nelspruit. The necrosis, stunting, mortality and coverage will be presented and discussed separately.

4.3.2.1.1. Necrosis

A thorough analysis of the 28 DAA data revealed that a significant interaction between treatments and water volume exists ($p<0.05$). A common trend surfaced that an increase in water volume led to an increase in necrosis (Figure 4.13).

Although there are some unexpected dips the general consensus is still that a higher water volume leads to a higher percentage necrosis as is evident in Figure 4.13. Destinaire™ and Summit Super (Treatments 4 and 5 respectively) are the adjuvants that conform most to this norm and also achieves, in general, quite high necrosis ratings when combined with quizalofop-p-tefuryl.

Treatment 4 contains Destinaire™ solo, with quizalofop-p-tefuryl, and Figure 4.13 shows a definite increase in necrosis due to an increase in water volume. The same can be said for Treatment 5 where Summit Super combined with quizalofop-p-tefuryl also achieved higher necrosis levels with an increase in water volume. Destinaire™ and Summit Super feature once again in causing high percentages of necrosis when in combination with Class Act NG™, in Treatments 9 and 10 respectively (Figure 4.13).

At the Nelspruit trial site there was substantially more necrosis caused by the lower water volumes. The increase of necrosis at the lower water volumes meant that a clearer picture was painted with regards to the amount of time take to reach the 80% necrosis threshold. Figure 4.14 and 4.15 both suggest that the 80% necrosis level was reached faster at 200 L ha^{-1}. Interlock™ solo with quizalofop-p-tefuryl (Treatment 7) provided the shortest time to reach the 80% necrosis threshold.

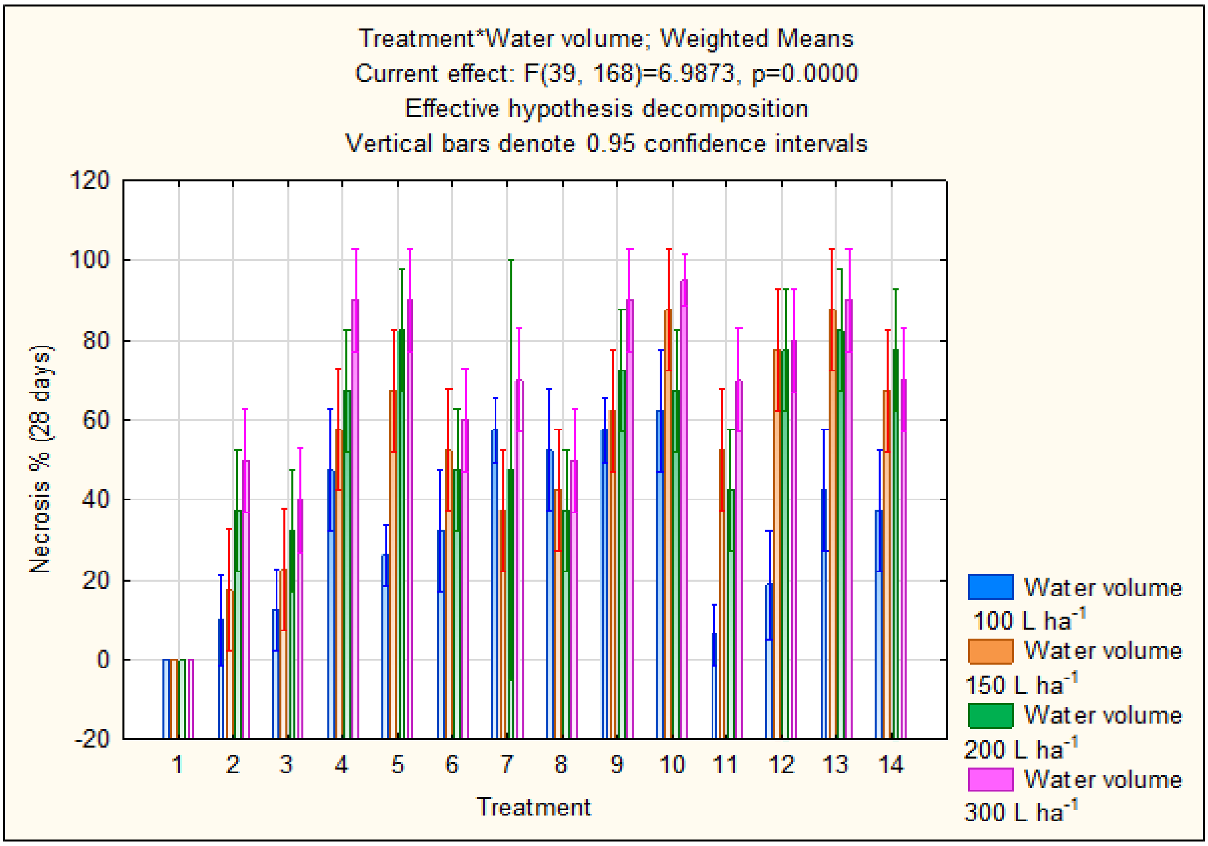


Figure 4.13: Effects of quizalofop-p-tefuryl in combination with various adjuvants on necrosis at 28 DAA, Nelspruit. (See Table 4.2 for treatment descriptions).

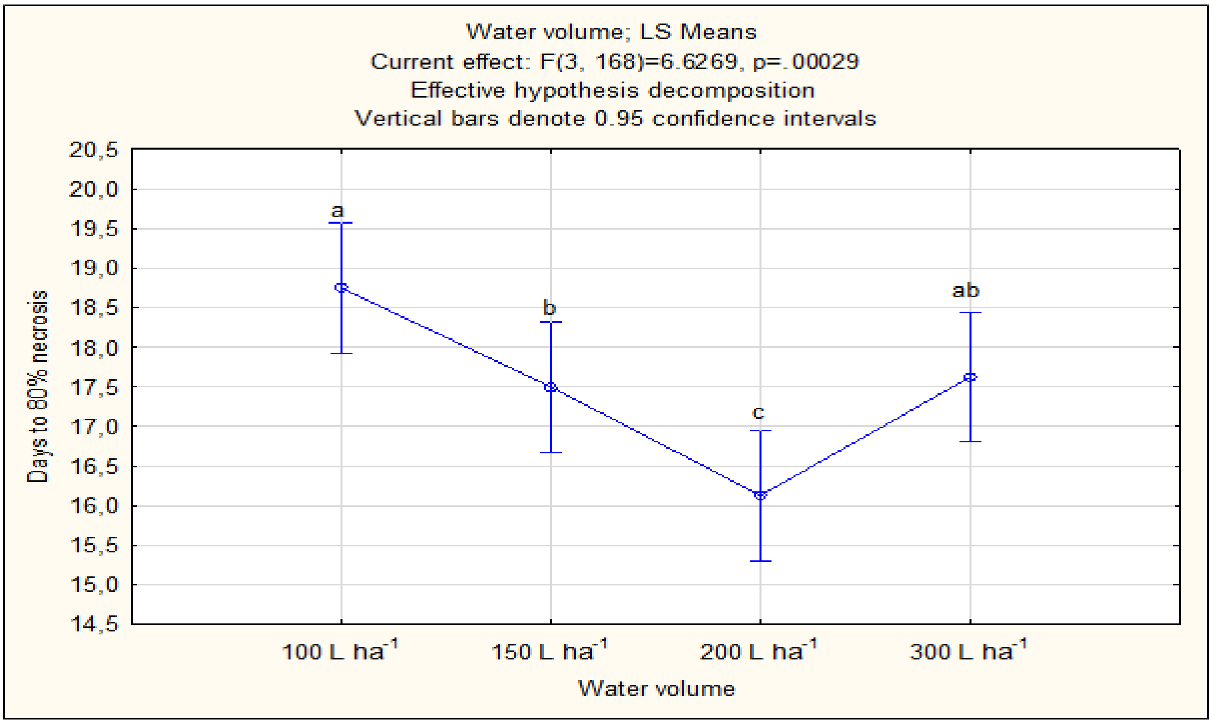


Figure 4.14: Time taken to reach the 80% necrosis threshold at different water volumes, Nelspruit. Different letters at data points indicate significant differences between treatments at $p = 0.05$.

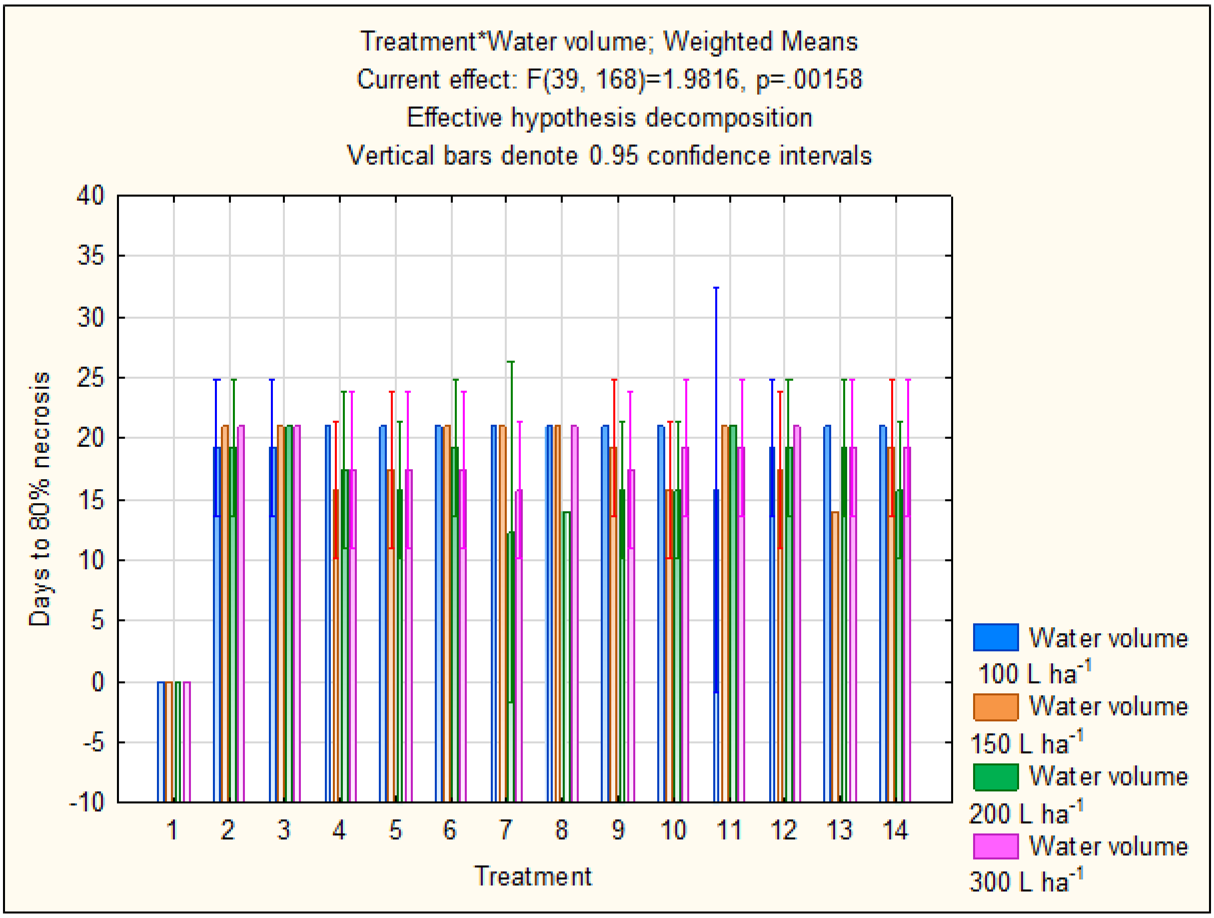


Figure 4.15: The effects of different adjuvants added to quizalofop-p-tefuryl on days to 80% necrosis threshold at 28 DAA, Nelspruit. (See Table 4.2 for treatment descriptions).

4.3.2.1.2 Stunting

The analysis of stunting data at 28 DAA revealed that treatments and water volumes caused a significant interaction ($p<0.05$). It appears that treatments had a profound effect in causing stunting.

There is a general trend that an increase in water volume led to an increase in stunting severity as is visible in Figure 4.16 and Figure 4.17. Another trend that arises is that treatments involving Summit Super seem to cause a more severe stunting at any water volume rate (Figure 4.17).

Treatments 5, 10 and 13 all contain Summit Super. As a solo adjuvant mixed with quizalofop-p-tefuryl, Summit Super (Treatment 5) produced the highest stunting severity of any solo adjuvant application. Combined with Class Act NG™ (Treatment 10) and Interlock™ (Treatment 13) in a mixture with quizalofop-p-tefuryl, Summit Super provided the highest overall stunting severity at 28 DAA (Figure 4.17).

Destinaire™ (Treatment 4) also provided fairly high stunting severities throughout the water volume range as is evident in Figure 4.17. As a solo adjuvant with quizalofop-p-tefuryl, Destinaire™ was only outperformed by Summit Super. When combined with Class Act NG™ and quizalofop-p-tefuryl (Treatment 9) and Interlock™ + quizalofop-p-tefuryl (Treatment 12), Destinaire™ was only outperformed by those same mixtures with Summit Super (Figure 4.17).

A lack of stunting at the lower water volumes led to an observation showing that the 80% stunting threshold was reached at a quicker tempo at the lower water volumes (Figure 4.15). At the higher water volumes all the treatments reached the 80% threshold at more or less 21 days where stunting was present (Figure 4.18).

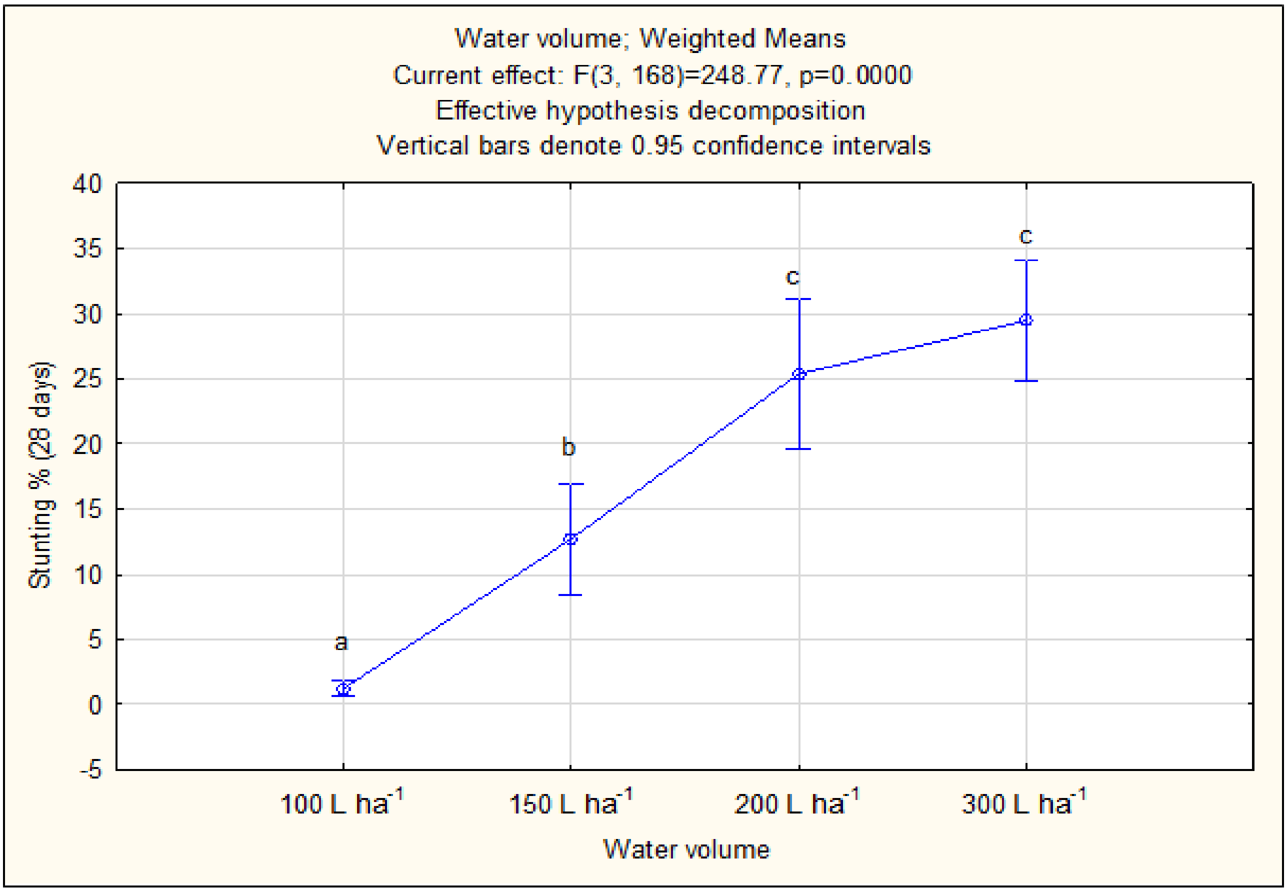


Figure 4.16: The effect of water volume on stunting of volunteer maize plants at 28 DAA, Nelspruit. (See Table 4.2 for treatment descriptions). Different letters at data points indicate significant differences between treatments at p = 0.05.

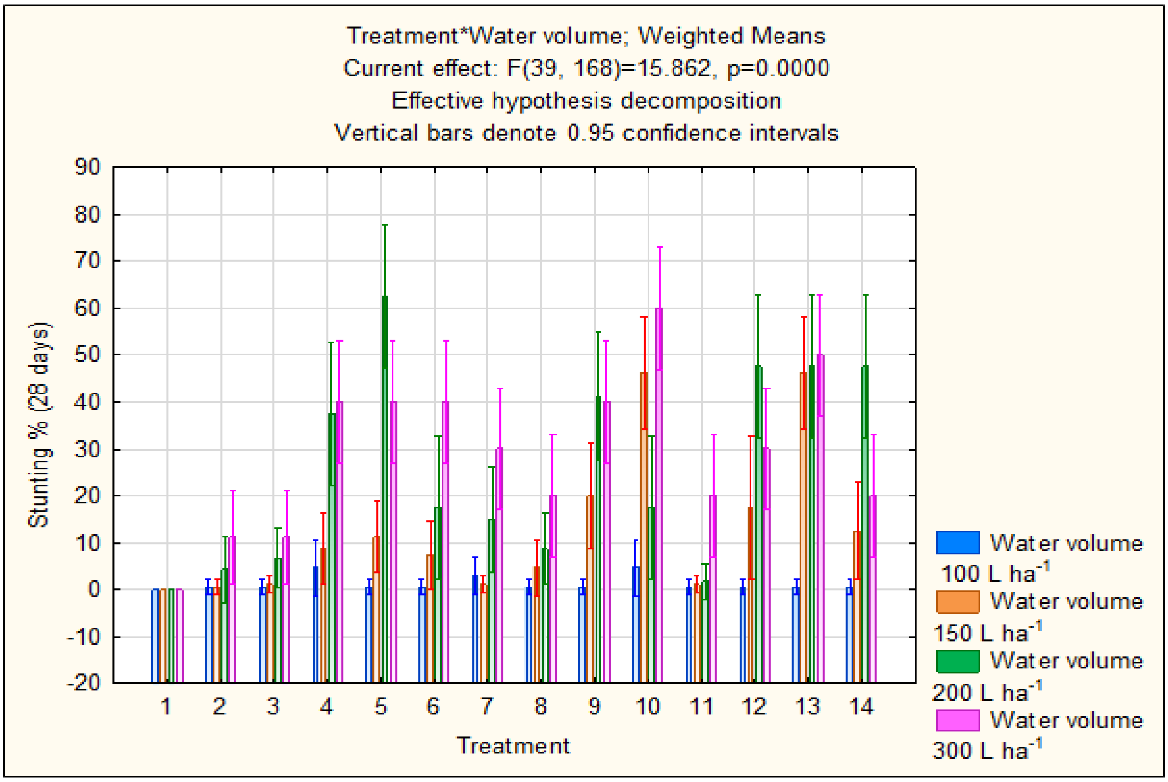


Figure 4.17: Effects of quizalofop-p-tefuryl in combination with various adjuvants on stunting at 28 DAA, Nelspruit. (See Table 4.2 for treatment descriptions).

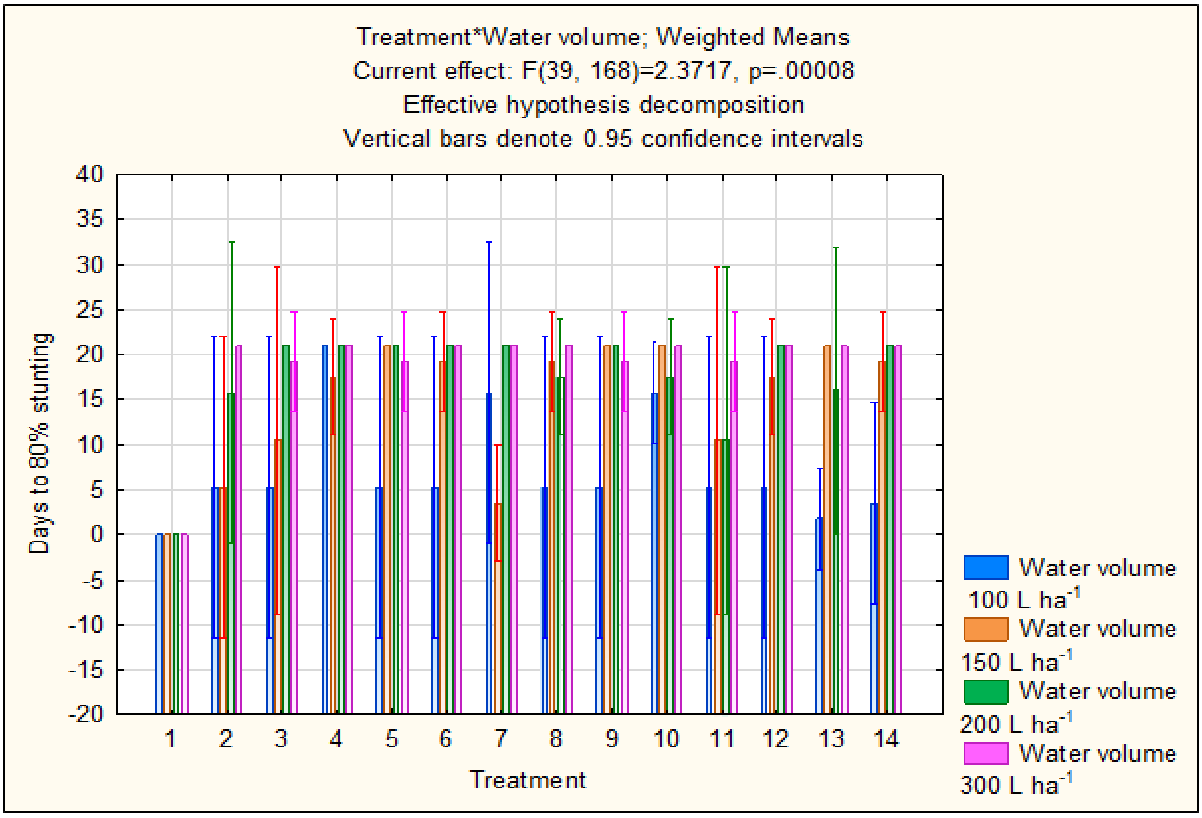


Figure 4.18: Number of days to 80% stunting threshold at 28 DAA, Nelspruit. (See Table 4.2 for treatment descriptions).

4.3.2.1.3 Mortality

Mortality data at 28 DAA suggests that 200 and 300 L ha^{-1} produced the highest mortality rates. At the Nelspruit site it appears that 200 liters per hectare achieved the highest mortality rate (Figure 4.19).

Destinaire™ (Treatment 4) solo with quizalofop-p-tefuryl as well as Summit Super solo and Class Act NG™ solo (Treatments 5 and 6 respectively) provided high mortality rates at 200 L ha^{-1}. Summit Super exhibited the highest mortality rate of the three with 100%, followed by Destinaire™ (97.5%) and Class Act NG™ (93.75%) when mixed solo with quizalofop-p-tefuryl (Figure 4.19).

The combination of Destinaire™ and Class Act NG™, with quizalofop-p-tefuryl (Treatment 9) proved to be very successful at 200 L $ha^{-1}$with a mortality rate of 98.75% (Figure 4.19). Successful combinations of Destinaire™ + Interlock™ (Treatment 12) and Summit Super + Interlock™ (Treatment 13) provided a mortality rate of 98.75% for both combinations (Figure 4.19).

A univariate test for significance revealed that there was no significant interaction between water volume and treatments to determine the 80% threshold for mortality. There were however significant differences between water volumes ($p<0.05$) in terms of days to reach the 80% mortality threshold (Figure 4.20).

A water volume of 150 L ha^{-1} appeared to achieve the 80% threshold the quickest at 14.25 days (Figure 4.20). This is a significant performance because at this measurement mortality was present at this lower water volume. The 100 L ha^{-1} followed with the second shortest amount of time to reach the 80% mortality threshold but this once again may be due to low mortality at 28 DAA at this water volume (Figure 4.19).

Water volumes of 200- and 300 L ha^{-1} both reached the 80% mortality threshold at 17 days which just resonates the previous statement that a higher water volume appear to be unwarranted (Figure 4.19).

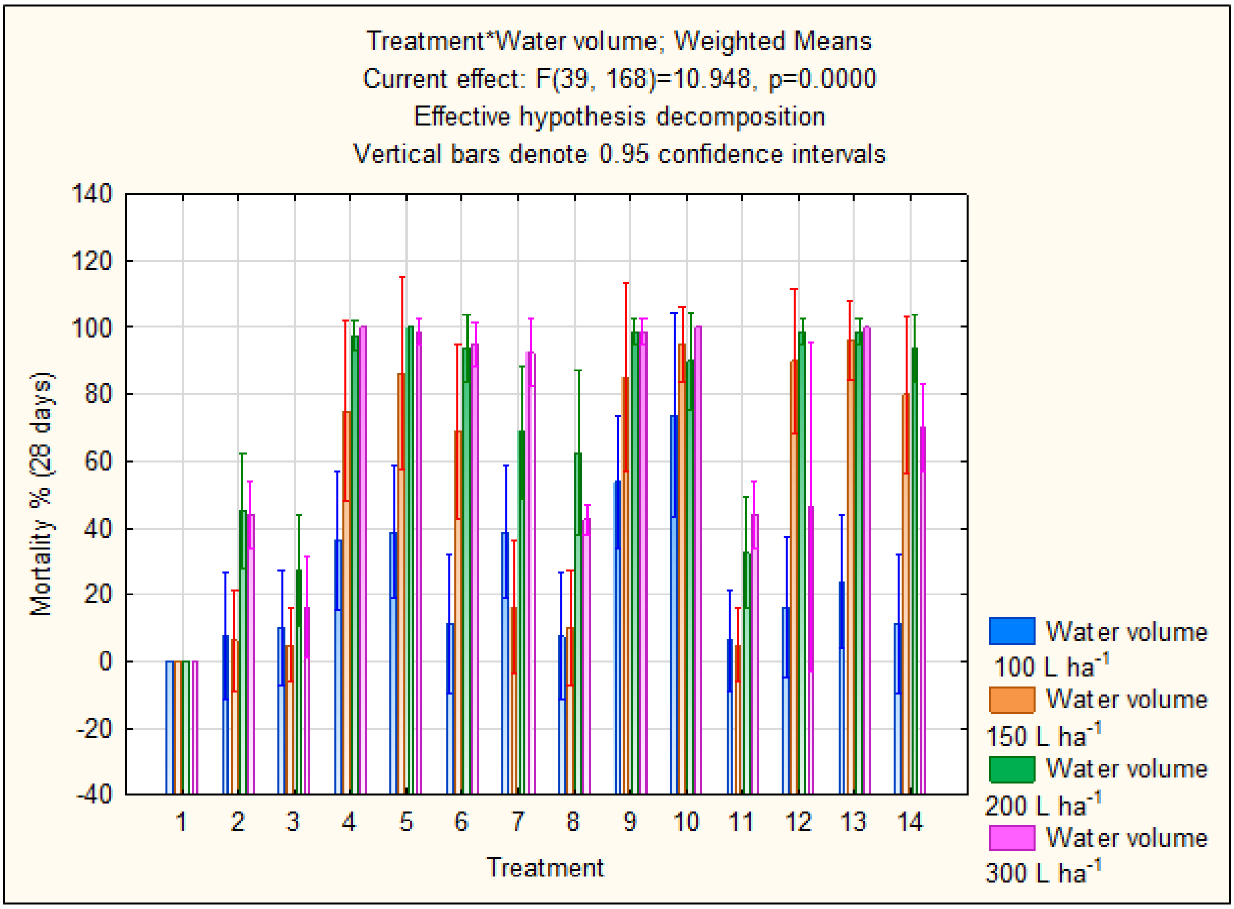


Figure 4.19: Effects of quizalofop-p-tefuryl in combination with various adjuvants on mortality of volunteer maize at 28 DAA, Nelspruit. (See Table 4.2 for treatment descriptions).

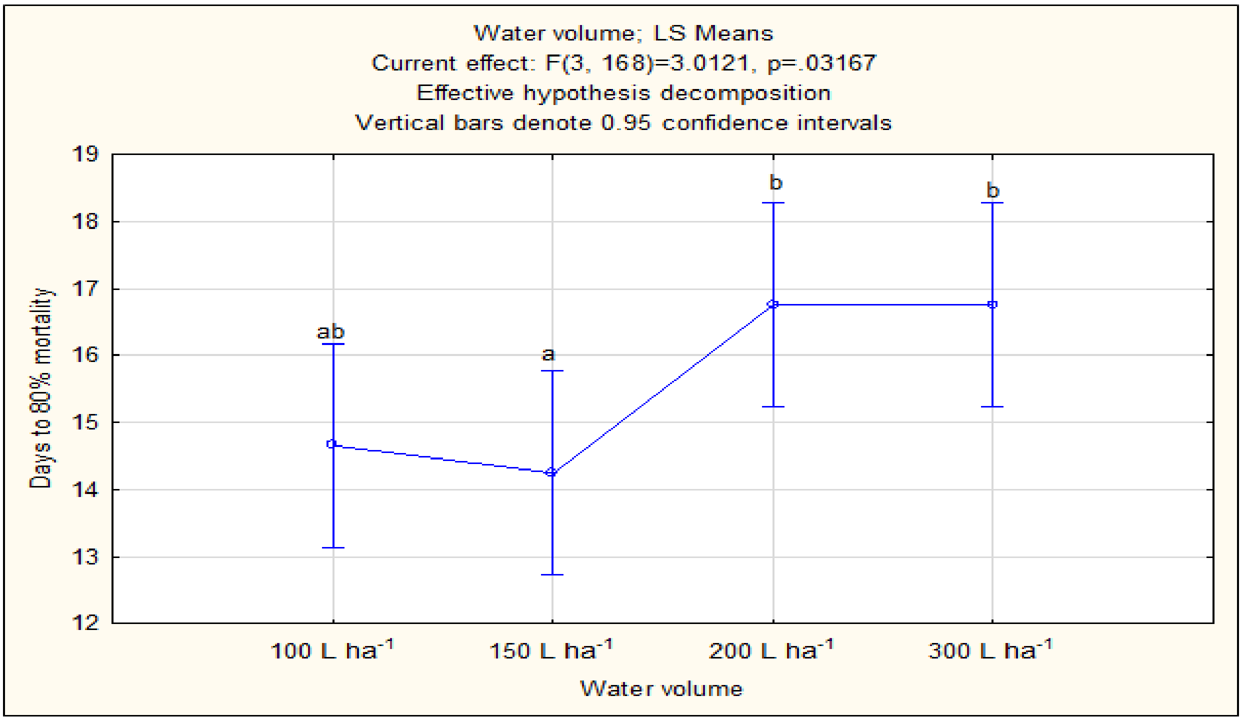


Figure 4.20: Number of days to 28 DAA 80% mortality threshold of volunteer maize at different water volumes, Nelspruit. Different letters at data points indicate significant differences between treatments at p = 0.05.

4.3.2.1.4 Coverage

The coverage analysis revealed that once again a significant interaction occurred between water volumes and treatments ($p<0.05$). The expected increase in water volume did exhibit a greater area coverage at 300 L ha^{-1} (Figure 4.21). The same however is not true for 200 L ha^{-1} which provided the lowest percentage area covered by the herbicide-adjuvant mixtures compared to 100, 150 and 300 L ha^{-1}.

At the lower water volumes, 100- and 150 L ha^{-1}, Treatment 10 covered the largest area (Figure 4.21). Treatment 10 consisted of Summit Super and Class Act NG™ featuring as the adjuvants combined with the herbicide quizalofop-p-tefuryl.

Water volumes of 300 L ha^{-1} once again provided the largest area coverage with the combination of Class Act NG™ + Interlock™ mixed with quizalofop-p-tefuryl (Treatment 14) covering 62% of the available area (Figure 4.21). Furthermore, Direct features for the first time by providing the second largest percentage coverage at 55.8% in combination with Class Act NG™ and quizalofop-p-tefuryl (Figure 4.21).

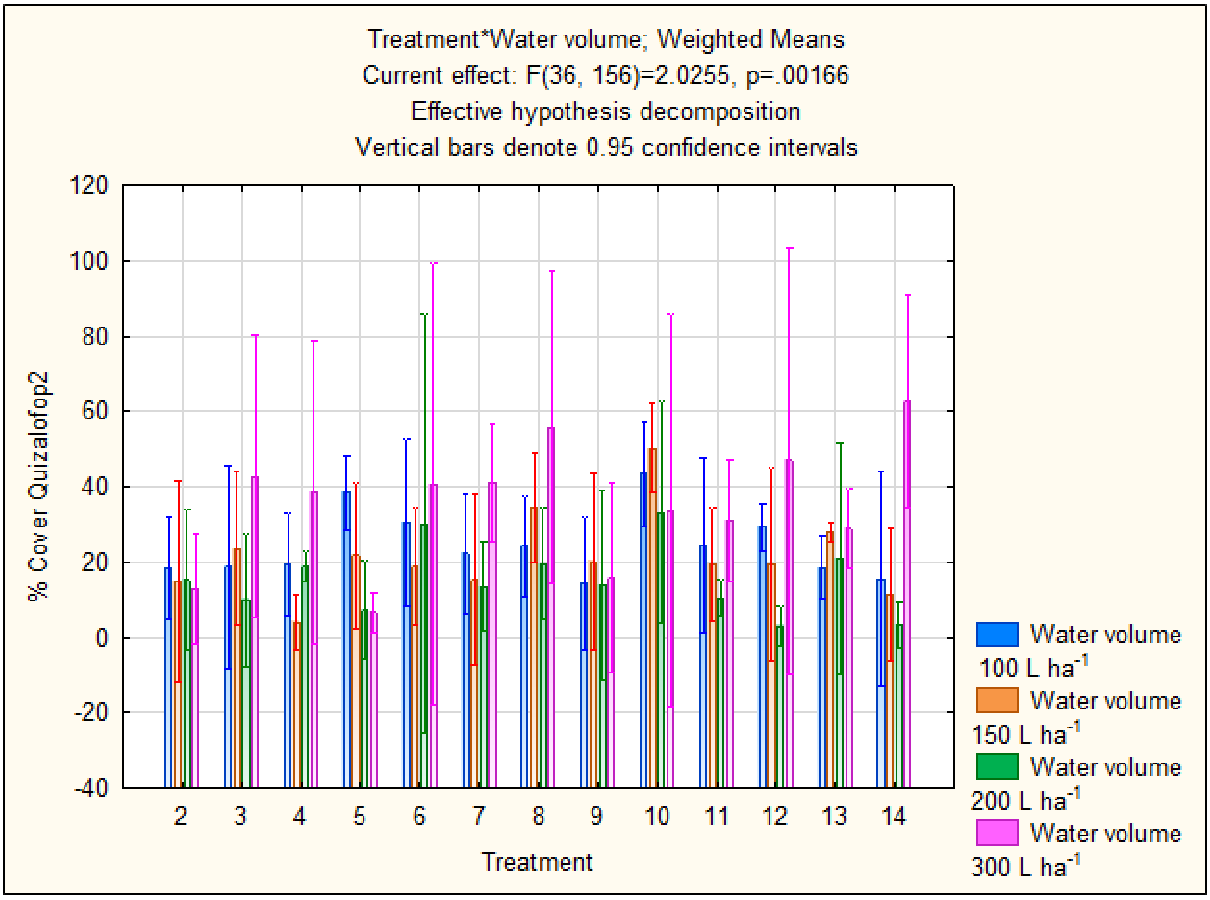


Figure 4.21: The effect of water volume on coverage of the herbicide-adjuvant mixtures on volunteer maize plants, Nelspruit.

4.3.2.2 Efficacy

The necrosis, stunting and mortality data at 28 DAA was used to determine the efficacy of herbicide-adjuvant mixtures to control glyphosate-resistant volunteer maize.

4.3.2.2.1 Necrosis

The efficacy trial at Nelspruit revealed a statistically significant difference between treatments (p<0.0.5). Summit Super performed extremely well and featured in the three treatments that statistically provided the highest necrosis percentages. Treatment 5 provided statistically more necrosis than any of the remaining solo adjuvant treatments (Figure 4.22). Treatment 10 caused similar necrosis readings to treatment 4 and contained Summit Super + Class Act NG™ + quizalofop-p-tefuryl (Figure 4.22). Treatment 13 contained Summit Super + Interlock™ + quizalofop-p-tefuryl and provided, statistically the highest necrosis percentage (Figure 4.22).

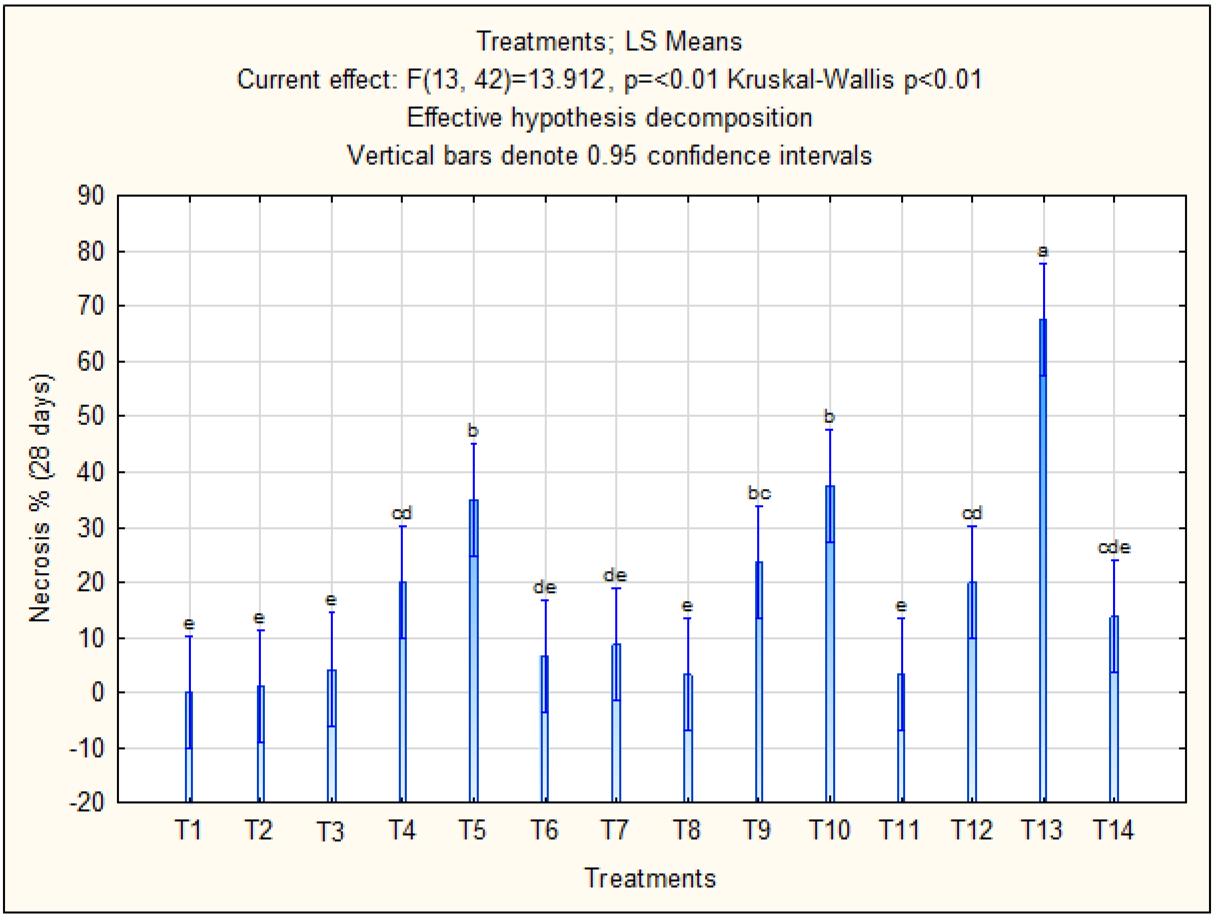


Figure 4.22: Effects of quizalofop-p-tefuryl in combination with various adjuvants on the efficacy to cause necrosis at 28 DAA, Nelspruit. (See Table 4.2 for treatment descriptions). Different letters at data points indicate significant differences between treatments at p = 0.05.

4.3.2.2.2 Stunting

When the stunting data is investigated a very similar trend to the necrosis arises. Treatment 13, Summit Super + Interlock™ + quizalofop-p-tefuryl, had statistically the highest stunting percentage at 10%. Treatment 10, Summit Super + Class Act NG™ + quizalofop-p-tefuryl, provided the second highest stunting percentage with 6.25% and did not differ from Treatment 5 which contains Summit Super solo with quizalofop-p-tefuryl (Figure 4.23).

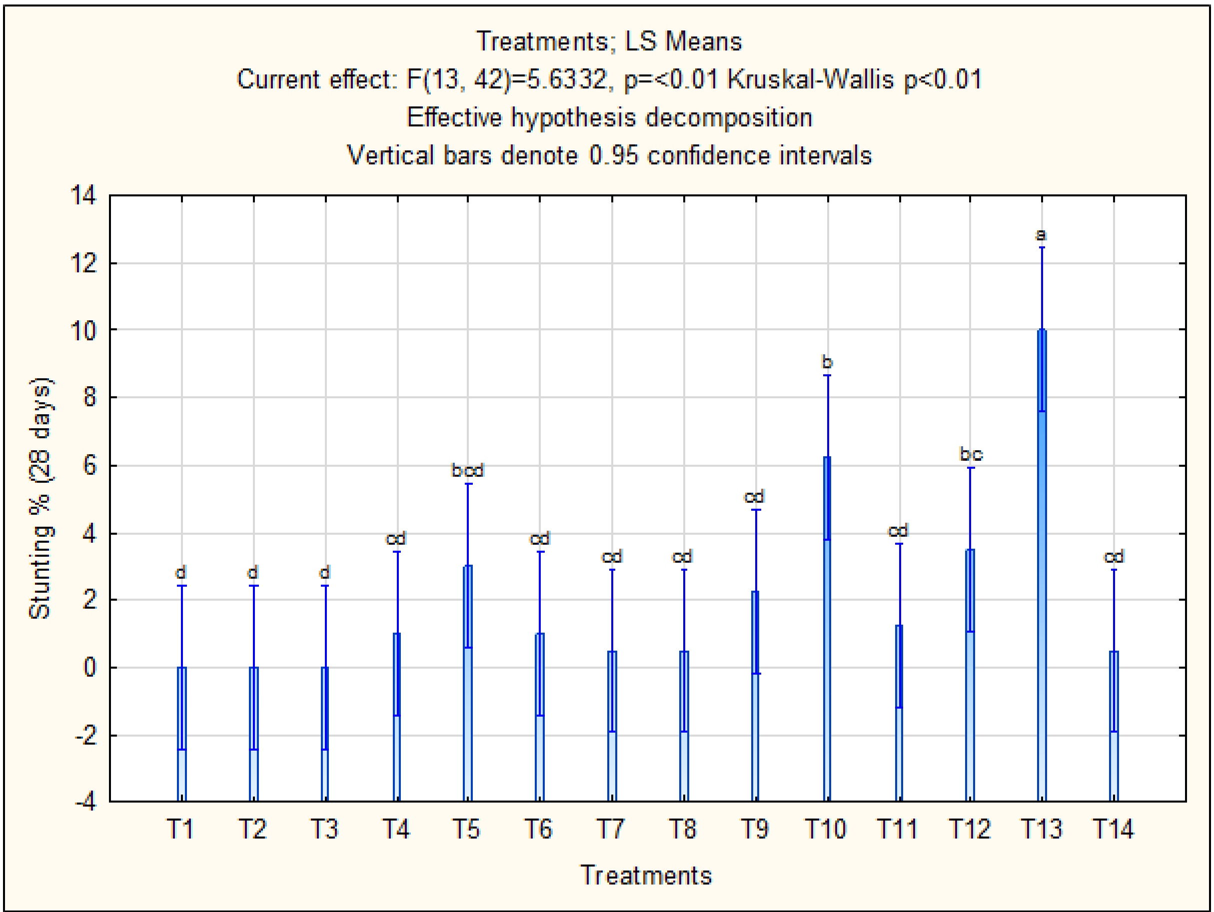


Figure 4.23: Effects of quizalofop-p-tefuryl in combination with various adjuvants on the efficacy to cause stunting at 28 DAA, Nelspruit. (See Table 4.2 for treatment descriptions). Different letters at data points indicate significant differences between treatments at p = 0.05.

4.3.2.2.3 Mortality

The same trend that arose in the stunting and necrosis evaluation continued in the mortality evaluation. Treatment 5 achieved a statistically higher mortality percentage than any of the remaining solo adjuvant treatments and most of the adjuvant combinations treatments (Figure 4.24). Treatment 13 provided, statistically, the highest mortality with 77.5% (Figure 4.24).

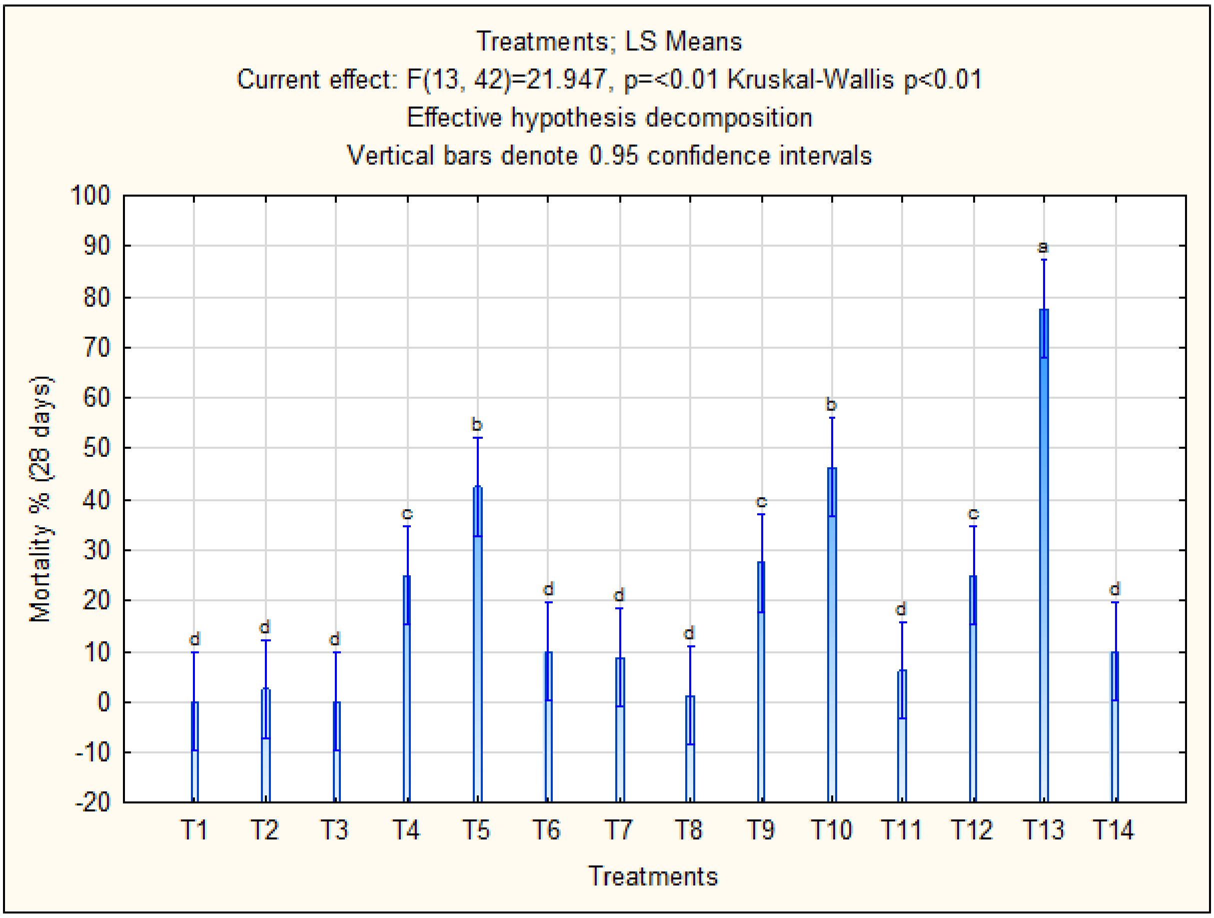


Figure 4.24: Effects of quizalofop-p-tefuryl in combination with various adjuvants on the efficacy to cause mortality at 28 DAA, Nelspruit. (See Table 4.2 for treatment descriptions). Different letters at data points indicate significant differences between treatments at p = 0.05.

4.4 Discussion

The deposition and efficacy trials at both trial sites delivered noteworthy results.

The first of those are the various observations made surrounding water volumes and the different influences these water volumes had on the efficacy of herbicide-adjuvant mixtures. The expected result of a direct correlation between water volumes and efficacy (Knoche 1994) did occur across most of the parameters and trials.

An increase in water volume to 300 L ha^{-1} produced an increase in necrosis and stunting severity as is evident in Figures 4.1, 4.4, 4.13 and 4.17. The increase of water volume to 300 L ha^{-1} also produced a higher mortality rate (Figure 4.6 and 4.19). The same trend continues when coverage is considered where the highest coverage percentages were achieved at 300 L ha^{-1} (Figure 4.8 and 4.21).

The second notable observation is the impact adjuvants have on the efficacy of quizalofop-p-tefuryl. The addition of adjuvants was expected to increase the efficacy of quizalofop-p-tefuryl (Beckett et al. 1992). This prediction and expectation were supported by the results obtained in this thesis.

At all the tested water volumes the addition of an adjuvant to quizalofop-p-tefuryl, except Direct, produced an increase in necrosis, stunting and mortality. This observation is true for the trial sites at both Morgenzon and Nelspruit. Destinaire™ solo with quizalofop-p-tefuryl proved to be the most successful solo adjuvant mixture with quizalofop-p-tefuryl at the Morgenzon trial site.

In Morgenzon, Destinaire™ was the adjuvant, in a solo mixture with quizalofop-p-tefuryl, that constantly provided the highest readings when evaluations were done. When necrosis is considered Destinaire™ caused the highest severity during the deposition trial (Figure 4.1) and the efficacy trial (Figure 4.10). Destinaire™ + quizalofop-p-tefuryl was also one of the high-ranking treatments in causing stunting during the deposition trial (Figure 4.11) and the solo adjuvant treatment that produced the highest stunting percentage during the efficacy trial (Figure 4.11). The mortality evaluations confirmed the necrosis and stunting evaluations, in that Destinaire™ greatly increased the efficacy of quizalofop-p-tefuryl to control glyphosate-resistant volunteer maize (Stougaard 1997). During the deposition trial the combination of Destiniare™ + quizalofop-p-tefuryl (Treatment 4) produced the highest mortality rate for any solo adjuvant mixture (Figure 4.6). This observation was repeated during the efficacy trial (Figure 4.12).

In Nelspruit Summit Super seemed to have the most profound effect on the efficacy of quizalofop-p-tefuryl to control glyphosate-resistant volunteer maize. During the deposition trial Summit Super + quizalofop-p-tefuryl (Treatment 5) was responsible for the highest necrosis percentage of any solo adjuvant mixture (Figure 4.13). The same observation was made when stunting is considered where Treatment 5 provided the highest percentage stunting during the deposition trial (Figure 4.17). The mortality evaluation for the deposition trial confirms that Summit Super compliments quizalofop-p-tefuryl in the control of glyphosate-resistant volunteer maize (Figure 4.19).

The efficacy trial in Nelspruit confirmed the observations that were made during the deposition trial where Treatment 5 produced higher percentages necrosis (Figure 4.22), stunting (Figure 4.23) and mortality (Figure 4.24) than any other of the solo adjuvant mixtures.

The reasons for the different adjuvants being effective at the different trial sites are most likely due to the prevailing weather conditions. Weather data obtained from the ARC was used to investigate the interaction between climate conditions and the efficacy of herbicide-adjuvant mixtures to control glyphosate-resistant volunteer maize.

At the Morgenzon trial site a higher average daily maximum relative humidity was measured during the trial period, November 2018-January 2019, compared to the data from the trial period in Nelspruit, December 2018-February 2019 (Annexure A). Plants growing at a higher relative humidity tends to develop a softer cuticle (Dominques et al. 2011).

Destinaire™ is a penetration agent and increases the efficacy of herbicides by softening the cuticle of the target weeds species and thus aiding in the penetration and absorption of herbicides leading to increased efficacy (Jordan et al. 2011). Due to the already softened cuticle of the volunteer maize plants, caused by the higher humidity, the assumption may be made that Destinaire™ succeeded in aiding quizalofop-P-tefuryl by facilitating the easier penetration of the already softened cuticle and thus leading to an increase in quizalofop-P-tefuryl efficacy in Morgenzon (Varanasi et al. 2016).

A decrease in humidity will lead to an increase in evaporation (Monteith 1965). The Nelspruit trial site showed a lower average daily maximum relative humidity compared to the Morgenzon trial site (Annexure A). Humectants are designed to increase herbicidal activity by slowing the evaporation rate of herbicides and thus leading to an increase in herbicide absorption (Tu and Randall 2003). Humectants make this possible by extracting moisture from the surrounding atmosphere and ensuring a higher humidity which leads to a decreased rate of drying off (Xu et al. 2010). Summit Super is a humectant and the ability to decrease the evaporation rate of herbicides led to an increase in efficacy at the Nelspruit trial site where a lower relative humidity was observed.

The deposition and efficacy trials both confirmed that adjuvants increased the efficacy of quizalofop-p-tefuryl to control glyphosate-resistant volunteer maize as is predicted by (Beckett et al 1994). The protocol used for quizalofop-p-tefuryl prescribes various adjuvant combinations along with quizalofop-p-tefuryl. The deposition trial at both trial sites implied that more than one adjuvant does not secure an increase in herbicide performance. When the efficacy trial is observed the opposite is evident.

During the efficacy trial at Morgenzon and Nelspruit increases in herbicide performance was observed when more than one adjuvant was mixed with quizalofop-p-tefuryl. The necrosis (Figure 4.10) and stunting (Figure 4.11) evaluations showed that Treatment 13, quizalofop-p-tefuryl + Summit Super + Interlock, had statistically higher percentages than the solo adjuvant mixtures in Morgenzon. This however was not observed during the mortality evaluation where no significant differences were observed between Treatment 13 and Treatment 4, quizalofop-p-tefuryl + Destinaire™ (Figure 4.12). This leads to the conclusion that in Morgenzon more than one adjuvant caused higher volunteer maize injury severity but did not supply an increase in the control of glyphosate-resistant volunteer maize. In Nelspruit

Treatment 13 provided statistically higher necrosis (Figure 4.22), stunting (Figure 4.23) and mortality (Figure 4.24) than any solo adjuvant mixture.

Coverage data obtained from both trial sites appears to indicate that water volume, rather than adjuvants are responsible for an increase in coverage due to an inconsistency by the treatments (Figure 4.8 and Figure 4.21). A worthwhile observation to be made here is that Interlock™ did not provide the expected increase in coverage at any of the water volumes.

From all the results the following conclusions can be made. An increase in water volume leads to an increase in necrosis, stunting, mortality and coverage (Qasem 2011). More than one adjuvant does appear to increase the efficacy of quizalofop-p-tefuryl to control glyphosate-resistant volunteer maize but the financial impact of such combinations needs to be investigated (Stougaard 1997). Due to the unknown financial implications of more than one adjuvant the conclusion may be made that Destinaire™ and Summit Super are the adjuvants that I would recommend to add to quizalofop-p-tefuryl to improve the efficacy of quizalofop-p-tefuryl and to control glyphosate-tolerant volunteer maize. This is especially true when one considers that quizalofop-p-tefuryl was applied at half the recommended dosage rate and was still able to control glyphosate-resistant volunteer maize at acceptable rates due to the addition of these two adjuvants.

4.5 References

Beckett TH, Stoller EW, Bode LE. 1992. Quizalofop and sethoxydim activity as affected by adjuvants and ammonium fertilizers. *Weed Technology* 40:12-19.

Dominguez E, Jesús C, Heredia A. 2011. An overview on plant cuticle biomechanics. *Plant Science* 181: 77-84.

Jordan T, Johnson B, Nice G. 2011. Adjuvants used with herbicides: factors to consider. Purdue Extension Weed Science. Available at https://ag.purdue.edu/btny/weedscience/Pages/default.aspx [accessed 17 September 2019].

Knoche M. 1994. Effect of droplet size and carrier volume on performance of foliage-applied herbicides. *Crop Protection* 13:163-178.

Monteith JL. 1965. Evaporation and environment. *Symposia of Experimental Biology* 19: 205-234.

Qasem JR. 2011. Herbicide applications: problems and considerations. In: Kortekamp A (eds), *Herbicides and Environment.* Shanghai: InTech. pp 643-664.

Stougaard RN. 1997. Adjuvant combinations with quizalofop for wild oat (*Avena fatua)* control in peppermint (*Mentha piperita*). *Weed Technology* 11: 45-50.

Tu M, Randall JM. 2003. Adjuvants. In: Tu M, Hurd C, Randall JM (eds), Weed Control Methods Handbook. Virginia: The Nature Conservancy.

Varanasi A, Vara Prasad PV, Jugulam M. 2016. Impact of climate change factors on weeds and herbicide efficacy. *Advances in Agronomy* 135: 107-138.

Xu L, Zhu H, Ozkan HE, Bagley WE, Krause CR. 2010. Droplet evaporation and spread on waxy and hairy leaves associated with type and concentration of adjuvants. Pest Management Science.

Chapter 5: Clethodim

5.1 Introduction:

Clethodim is an ACCase inhibitor herbicide used to control glyphosate-resistant volunteer maize. To determine the influence of adjuvants on the efficacy of clethodim to control glyphosate-resistant volunteer maize, the herbicide was combined with five different adjuvants in different combinations (Table 5.2).

The herbicide-adjuvant mixtures were then tested in a deposition and efficacy trial to determine the influence of these adjuvants on the efficacy of clethodim to control the volunteer maize. The deposition trial consisted of necrosis, stunting, mortality and coverage data at different water volumes. The efficacy trial consisted of necrosis, stunting and mortality data at a constant water volume.

5.2 Materials and Methods:

The materials and methods as discussed in Chapter 3 is applicable to clethodim. In this chapter the spraying protocol, which illustrates the different treatment combinations, will be presented. This protocol is the same for both the deposition and efficacy trials.

5.2.1 Protocol

As discussed in Chapter 2, five adjuvants were employed to test the impact adjuvants have on the efficacy of herbicides. In Chapter 2 the five adjuvants were identified as a surfactant/oil adjuvant combination, a surfactant/fertilizer combination, a high surfactant oil concentrate (HSOC) methylated seed oil, a liquid AMS/surfactant/humectant combination and a deposition agent. The adjuvants will be discussed by referring to the product names of these adjuvants (Table 5.1). The product Clethodim was used as the active ingredient clethodim.

Table 5.1: Adjuvants and trade names

Adjuvant	Product name
Surfactant/oil adjuvant combination	Direct
A high surfactant oil concentrate (HSOC) methylated seed oil	Destinaire™
A surfactant/fertilizer combination	Summit Super
A liquid AMS/surfactant/humectant combination	Class Act NG™
Deposition agent	Interlock™

The treatment protocol is indicated in table 5.2 and illustrates the different Clethodim and adjuvant combinations. It is worth mentioning again that the rate used for Clethodim is half the rate prescribed by the product label. This was done to exaggerate the adjuvant influence on the efficacy of the herbicides.

Table 5.2: Treatment protocol

Treatment	**Product combination**	**Dosage rate:** l ha^{-1}; v/v
1	Untreated control (UTC)	
2	Clethodim	0.5
3	Clethodim + Direct	0.5 + 0.1%
4	Clethodim + Destinaire™	0.5 + 1
5	Clethodim + Summit Super	0.5 + 0.3%
6	Clethodim + Class Act NG™	0.5 + 2%
7	Clethodim + Interlock™	0.5 + 0.3
8	Clethodim + Direct + Class Act NG™	0.5 + 0.1% + 2%
9	Clethodim + Destinaire™+ Class Act NG™	0.5 + 1 + 2%
10	Clethodim + Summit Super + Class Act NG™	0.5 + 0.3% + 2%
11	Clethodim + Direct + Interlock™	0.5 + 0.1% + 0.3
12	Clethodim + Destinaire™+ Interlock™	0.5 + 1 + 0.3
13	Clethodim + Class Act NG™ + Interlock™	0.5 + 0.3% + 0.3

The results for clethodim will be discussed under the following headings: Deposition and efficacy. The deposition results are based on the data retrieved from applying the treatments at 100, 150, 200 and 300 L water ha^{-1}. Coverage data for the deposition trial was obtained from analyzing the water sensitive paper that was placed in the deposition plots with the purpose of examining the influence that water volume and adjuvants have on the coverage of herbicides. The efficacy results are based on the efficacy trial done where the treatments were applied at 200 L water ha^{-1}.

The deposition and efficacy will be discussed separately for each trial site. The Morgenzon trial site will be referred to as Trial site 1 and the Nelspruit trial site will be referred to as Trial site 2.

5.3 Results

5.3.1 Morgenzon

The trial site at Morgenzon was used for a deposition and efficacy trial and the results from that trial site will be presented here.

5.3.1.1 Deposition

The necrosis, stunting and mortality data from 28 days after application (DAA) was used to investigate the impact of different water volumes on the efficacy of herbicide-adjuvant mixtures.

Necrosis, stunting, mortality and coverage results will be presented and discussed separately.

5.3.1.1.1 Necrosis

Necrosis at 28 DAA revealed a significant interaction between water volume and treatments ($p<0.05$). A water volume of 300 L ha^{-1}provided an increase in necrosis severity (Figure 5.1). The remaining water volumes are difficult to separate due to an inconsistent trend within these water volumes. The poor performance, compared to 100 and 150 L ha^{-1}, by treatments at 200 L ha^{-1} is cause for concern as this is the prescribed water volume for clethodim.

Destinaire™ solo (Treatment4) with clethodim provided the highest necrosis percentages when compared to the other solo herbicide-adjuvant mixtures at 100,150 and 300 L ha^{-1} (Figure 5.1). When applied at 300 L ha^{-1} Destinaire™ + clethodim provided a necrosis percentage of 97.5% which is the highest for any of the treatments. At 100 L ha^{-1} the Destinaire™ and clethodim combination supplied a necrosis percentage of 82.5% and at 150 L ha^{-1} a necrosis measurement of 85% was reached (Figure 5.1). Also, worth mentioning is that Treatment 8, Direct + Class Act™ + clethodim provided more than 90% necrosis when applied at 100 L ha^{-1}.

The 80% threshold analysis revealed that 300 L ha^{-1} generally reached the threshold the quickest, followed by 150 L ha^{-1} (Figure 5.2). At 150 L ha^{-1} Summit Super + clethodim reached the 80% threshold in the least amount of time of 14 days (Figure 5.2). Summit Super, Class Act NG™ and Interlock™ reached the 80% threshold the quickest of the solo treatments, when combined with clethodim, at approximately 16 days (Figure 5.2).

These observations are significant due to the presence of necrosis at all the water volumes. From the necrosis and time evaluation it is evident that 150 L ha^{-1} still proves effective in causing volunteer maize injury. At 300 L ha^{-1} however, the necrosis was substantially higher than at the prescribed water volume of 200 L ha^{-1}. The time taken to produce 80% volunteer maize injury also revealed that 300 L ha^{-1} will injure the volunteer maize plant at a higher rate.

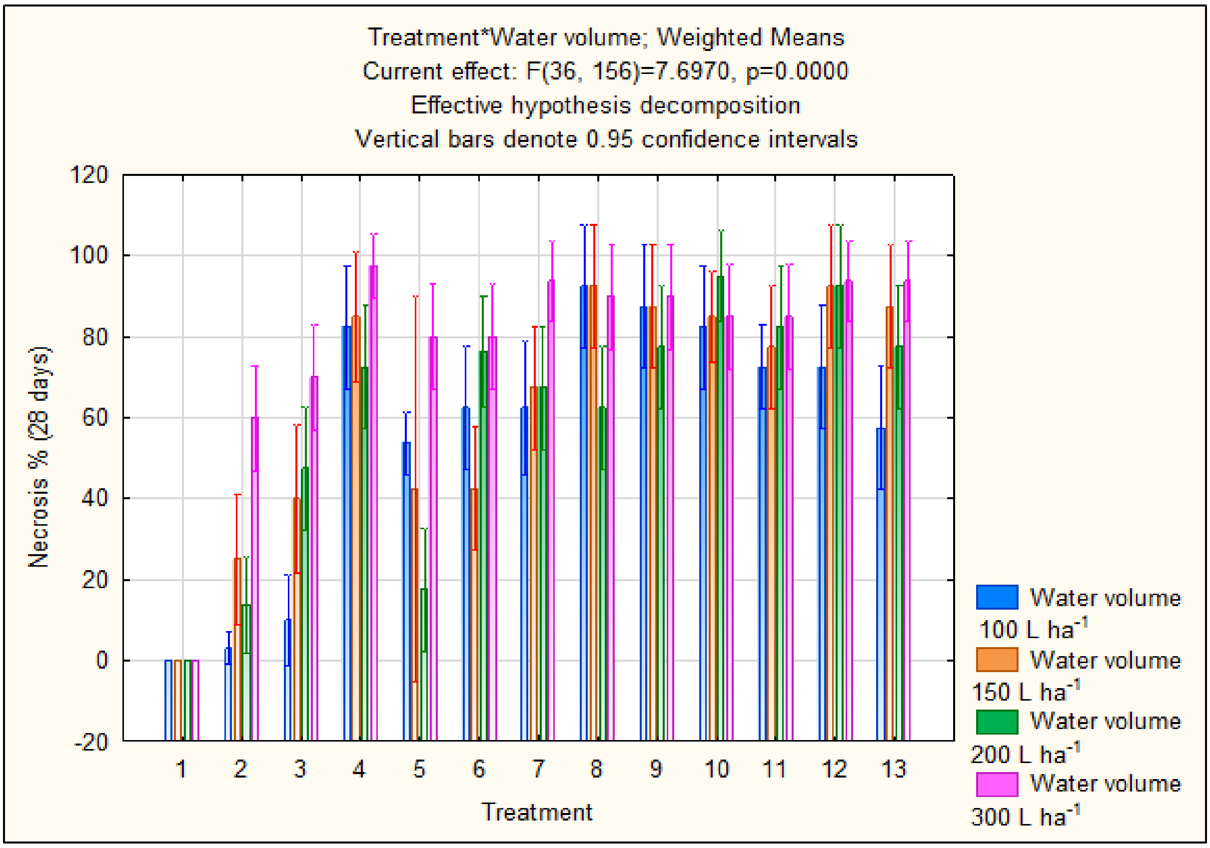


Figure 5.1: Effects of clethodim in combination with various adjuvants on necrosis of volunteer maize plants at 28 DAA, Morgenzon. (See Table 5.2 for treatment descriptions).

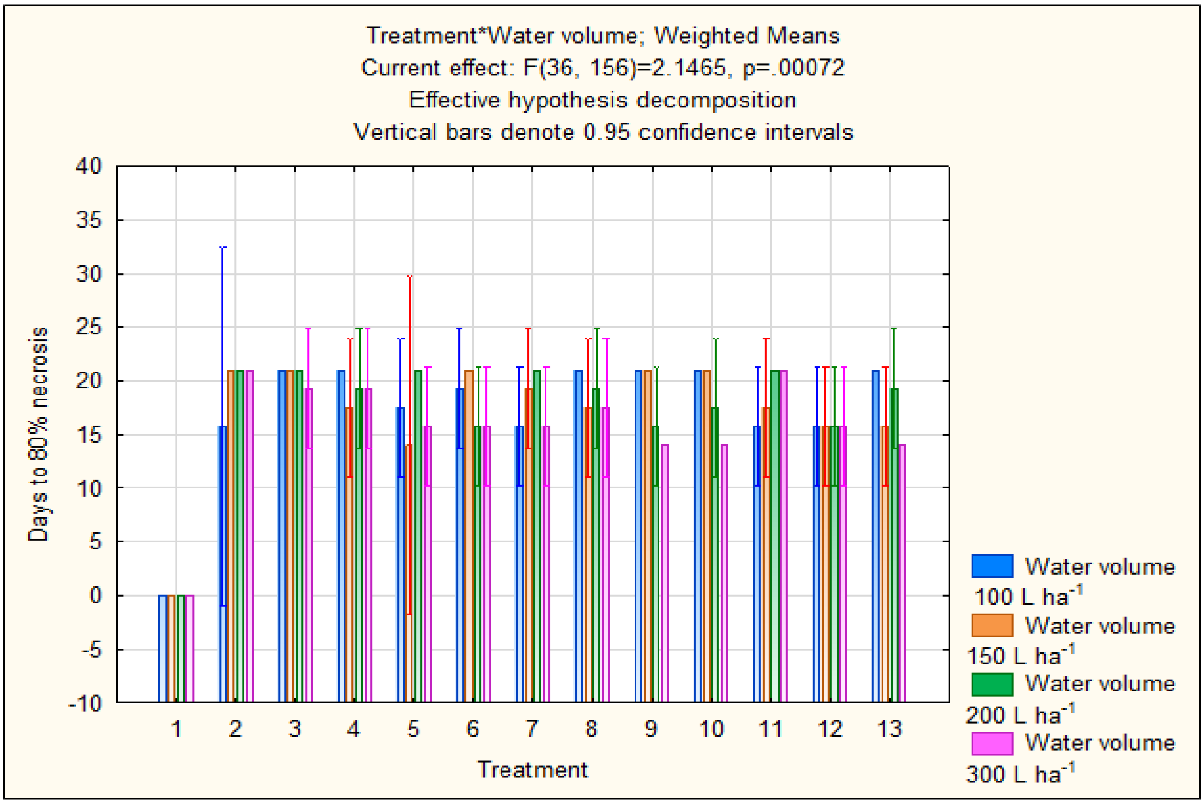


Figure 5.2: The effects of different adjuvants added to clethodim on days to 80% necrosis threshold of volunteer maize plants at 28 DAA, Morgenzon. (See Table 5.2 for treatment descriptions).

5.3.1.1.2 Stunting

A significant p value (p<0.05) was obtained when the influence of treatments and water volume was analysed to evaluate their effect on stunting caused by adjuvant mixtures with clethodim.

The first observation is that higher water volumes (200 and 300 L ha^{-1}) resulted in higher stunting ratings with the exception of Treatments 5 and 8 where 200 L ha^{-1} caused relatively low stunting percentages (Figure 5.3). The 300 L ha^{-1} treatments proved to cause the most severe stunting followed by 200 L ha^{-1}. Destinaire™ (Treatment 4) generally provided the highest stunting severity when combined with clethodim across the water volume range for solo adjuvants (Figure 5.3). Adjuvant mixtures that caused the most severe stunting also contained Destinaire™. At 150, 200 and 300 L ha^{-1} Treatment 12 caused the most severe stunting at 52.5%, 56.25% and 55% respectively (Figure 5.3). Treatments 12 features Destinaire™ + Interlock™ combined with clethodim. Destinaire™ mixed with clethodim also caused the most severe stunting at 100 L ha^{-1} with 50% compared to the remaining solo adjuvants + clethodim combinations (Figure 5.3).

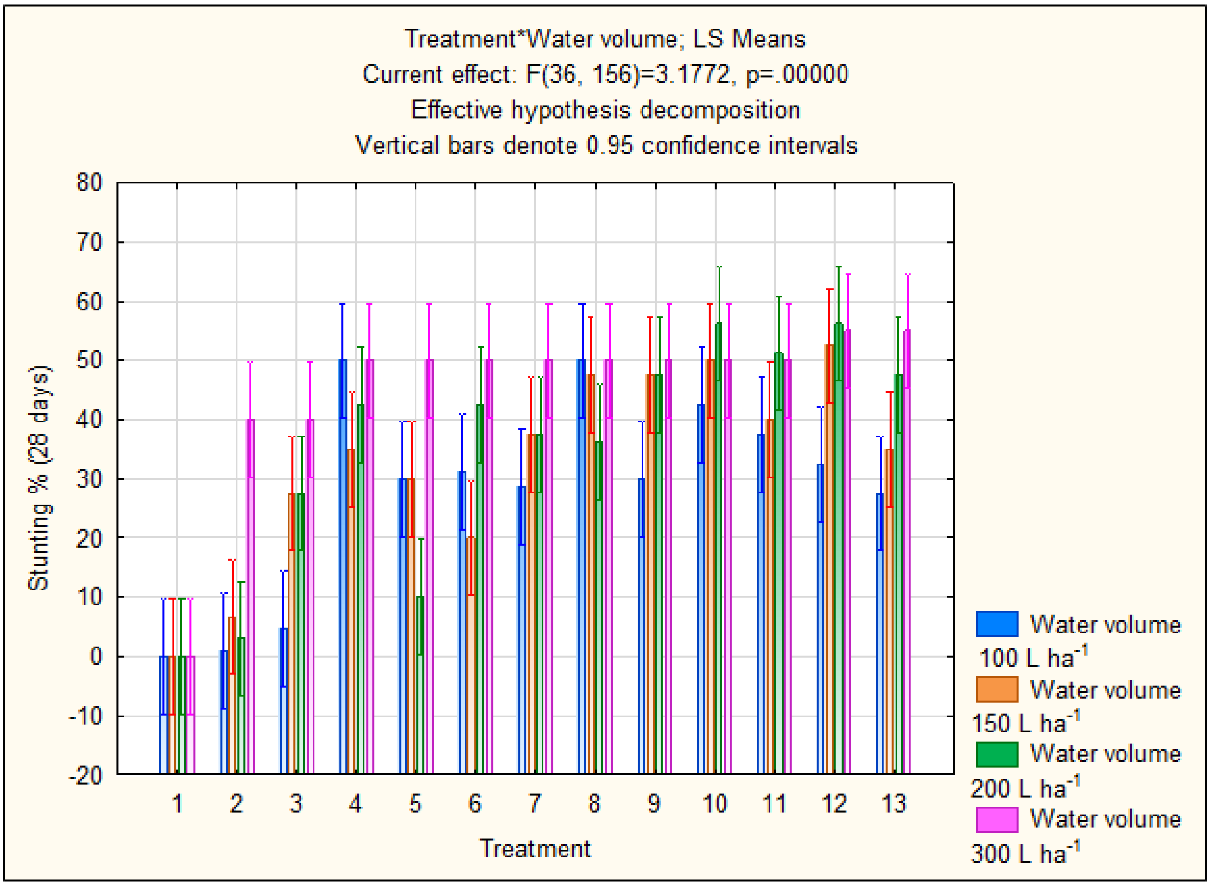


Figure 5.3: Effects of clethodim in combination with various adjuvants on stunting of volunteer maize plants at 28 DAA, Morgenzon. (See Table 5.2 for treatment descriptions).

The 80% stunting threshold revealed that no significant differences occurred between water volumes, nor significant interactions between treatments and water volumes. The only significant observation was between treatments ($p<0.05$). It was revealed that Treatment 2 reached the 80% stunting threshold the quickest at approximately 15 days. The remaining treatments all reached the threshold between 18 and 21 days (Figure 5.4). This can be attributed to an absence of eventual stunting at 28 DAA by Treatment 2 (Figure 5.3).

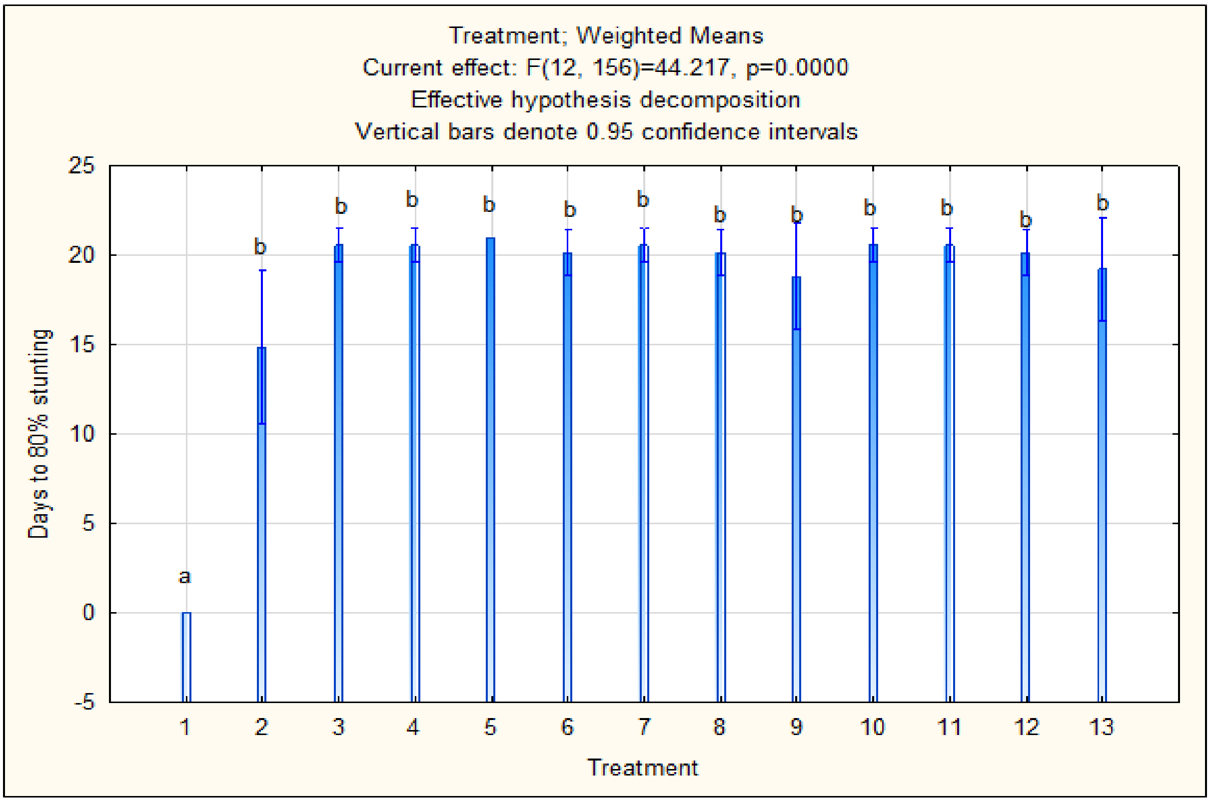


Figure 5.4: The effects of different adjuvants added to clethodim on days to 80% stunting threshold of volunteer maize plants at 28 DAA, Morgenzon. (See Table 5.2 for treatment descriptions). Different letters at data points indicate significant differences between treatments at p = 0.05.

5.3.1.1.3 Mortality

A univariate test of significance for mortality revealed a significant statistical interaction ($p<0.05$) between water volumes and treatments. The observation that a higher water volume has a more profound effect on the volunteer maize is also valid here as well. At 300 L ha^{-1} the highest mortality rates were observed (Figure 5.5). Similar to the necrosis evaluation, 150 L ha^{-1} outperformed 200 L ha^{-1} in causing mortality (Figure 5.5).

Destinaire™ applied solo with clethodim (Treatment 4) outperformed not only the other solo adjuvant + clethodim mixtures, but also most of the combined adjuvant mixtures in causing mortality of volunteer maize (Figure 5.5). At 100 L ha^{-1} Destinaire™ achieved 90% mortality, 150 L ha^{-1} resulted in 94.6% mortality, 200 L ha^{-1} caused 98.75% mortality and at 300 L ha^{-1} it resulted in 97.5% mortality. At 200 L ha^{-1} clethodim without an adjuvant achieved 12.5% mortality which is statistically less than Destinaire™ + clethodim which achieved a mortality rate of 98.75% (Figure 5.5). Destinaire™ in combination with Class Act™ (Treatment 9) also resulted in almost 100% mortality at all water volumes.

The 80% mortality threshold revealed that there were only significant differences to be found between treatments ($p<0.05$). Treatment 5 was the slowest to the 80% threshold at ± 19 days (Figure 5.6). This was significantly slower than treatments 4 (±15 days), 7 (±15 days) and 9 (±15 days), 12 (± 15 days) and 13 (± 15 days).

5.3.1.1.4 Coverage

A significant interaction was observed between water volumes and treatments ($p<0.05$). The first observation to be made is that the prescribed water volume of 200 L ha^{-1} provided the highest percentage cover (Figure 5.7). Treatment 3, Direct + clethodim, and Treatment 6, Class Act NG™ + clethodim, provided the highest percentage cover for the solo adjuvant mixtures (Figure 5.7). Treatment 10, Summit Super + Class Act NG™ + clethodim, caused the highest percentage cover for the adjuvant combination treatments at 300 L ha^{-1} water volumes (Figure 5.7).

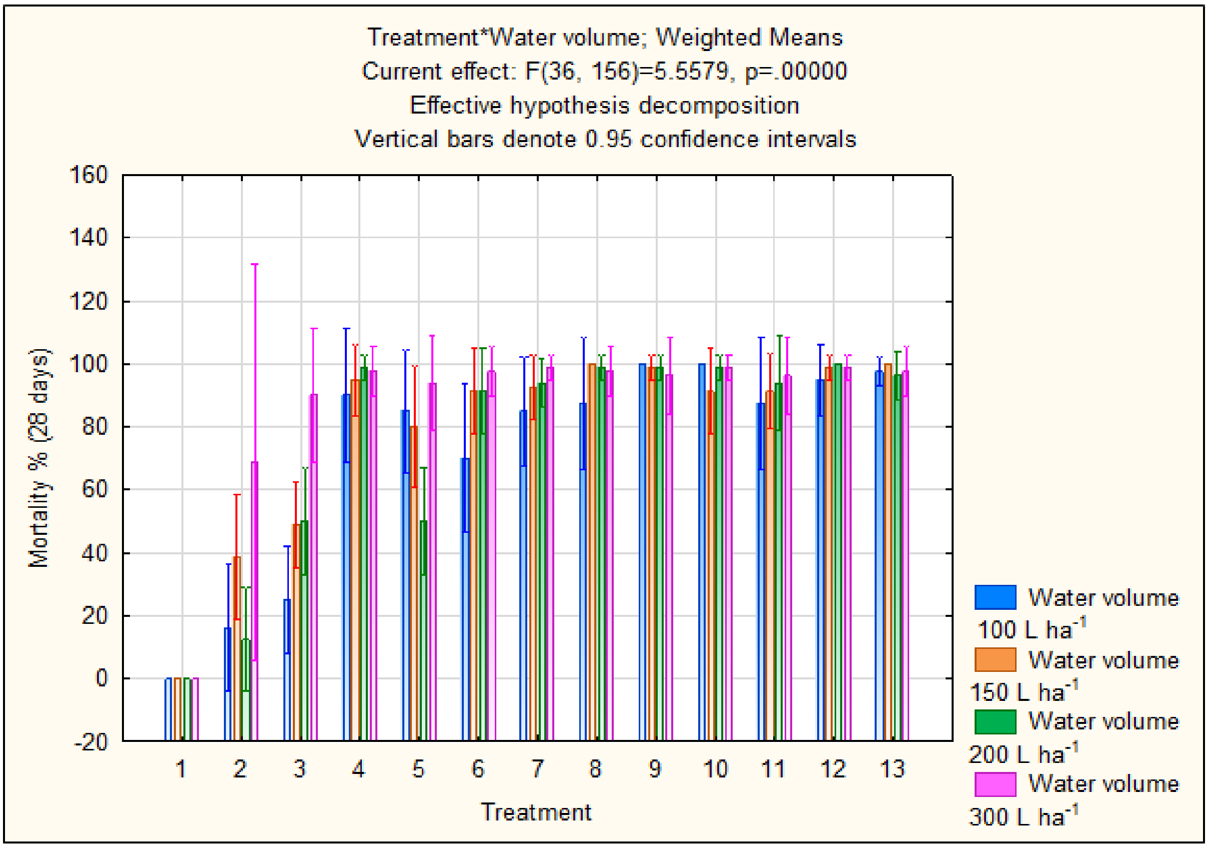


Figure 5.5: Effects of clethodim in combination with various adjuvants on percentage mortality of volunteer maize plants at 28 DAA, Morgenzon. (See Table 5.2 for treatment descriptions).

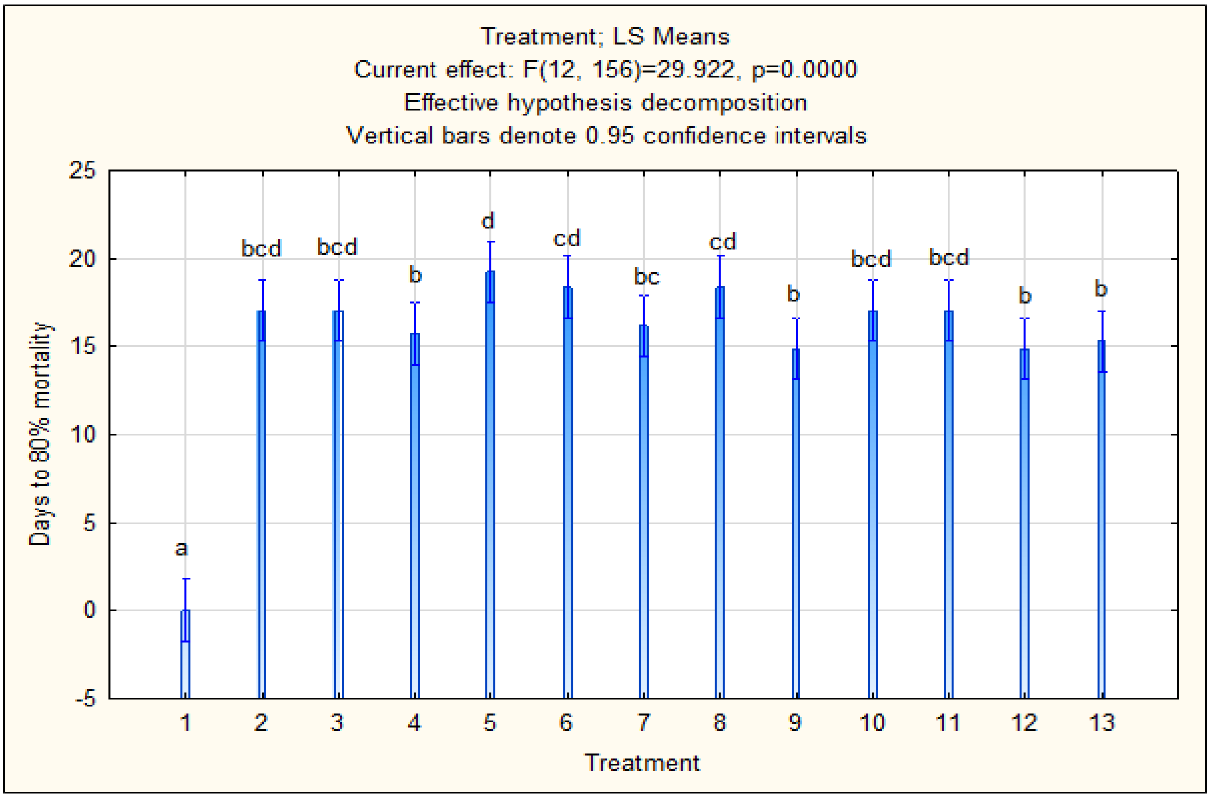


Figure 5.6: The effects of different adjuvants added to clethodim on days to 80% mortality threshold of volunteer maize plants at 28 DAA, Morgenzon. (See Table 5.2 for treatment descriptions). Different letters at data points indicate significant differences between treatments at p = 0.05.

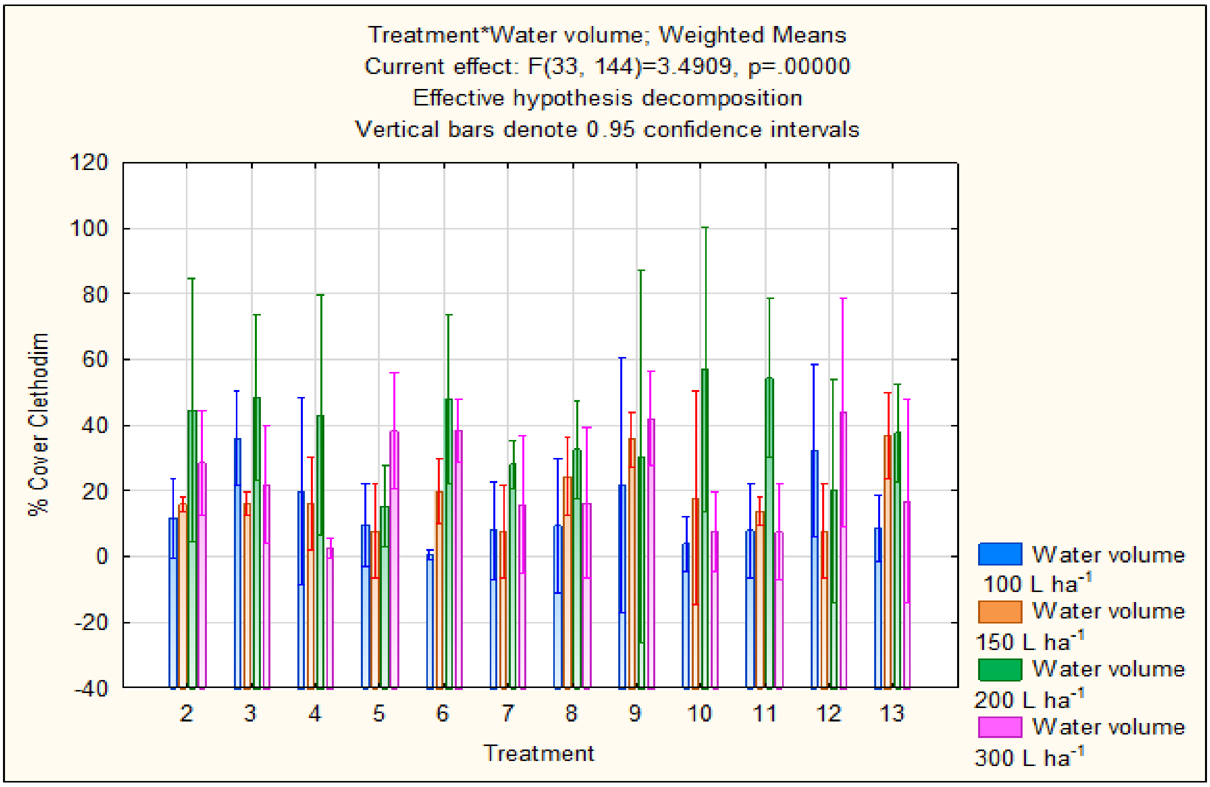


Figure 5.7: The effect of water volume on coverage of the herbicide-adjuvant mixtures on volunteer maize plants, Morgenzon. (See Table 5.2 for treatment descriptions).

5.3.1.2 Efficacy

The necrosis, stunting and mortality data at 28 DAA was used to determine the efficacy of herbicide-adjuvant mixtures to control glyphosate-resistant volunteer maize.

5.3.1.2.1 Necrosis

Significant differences were observed between treatments (p<0.05). Destinaire™ solo with clethodim (Treatment 4), caused a statistically higher necrosis percentage compared to most of the other solo adjuvant treatments, except Treatment 7 (Figure 5.8). Destinaire™ solo also achieved the highest overall necrosis with 92.5%. When the combination adjuvant treatments are observed, Destinaire™ features again in Treatments 9 and 12 which provided the highest necrosis percentages of all the adjuvant combination treatments (Figure 5.8). Treatment 9 consists of Destinaire™ + Class Act NG™ + clethodim and Treatment 12 consists of Destinaire™ + Interlock™ + clethodim (Figure 5.8).

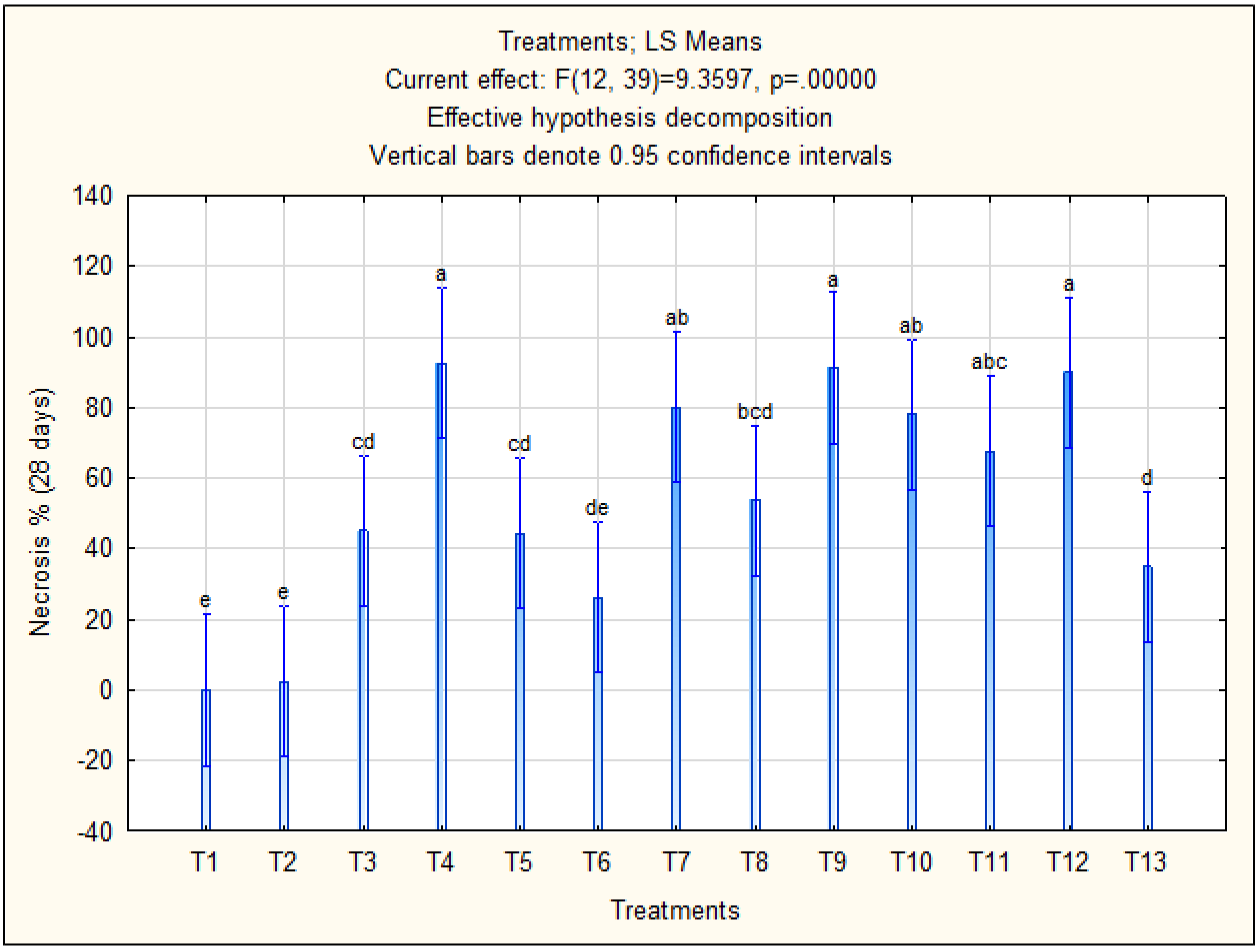


Figure 5.8: Effects of clethodim in combination with various adjuvants on necrosis of volunteer maize plants at 28 DAA, Morgenzon. (See Table 5.2 for treatment descriptions). Different letters at data points indicate significant differences between treatments at p = 0.05.

5.3.1.2.2 Stunting

The same trend occurs when stunting is evaluated as was observed during the necrosis evaluation. Destinaire™ features in the three treatments which provided the highest stunting percentage- Treatments 4, 9 and 12 (Figure 5.9).

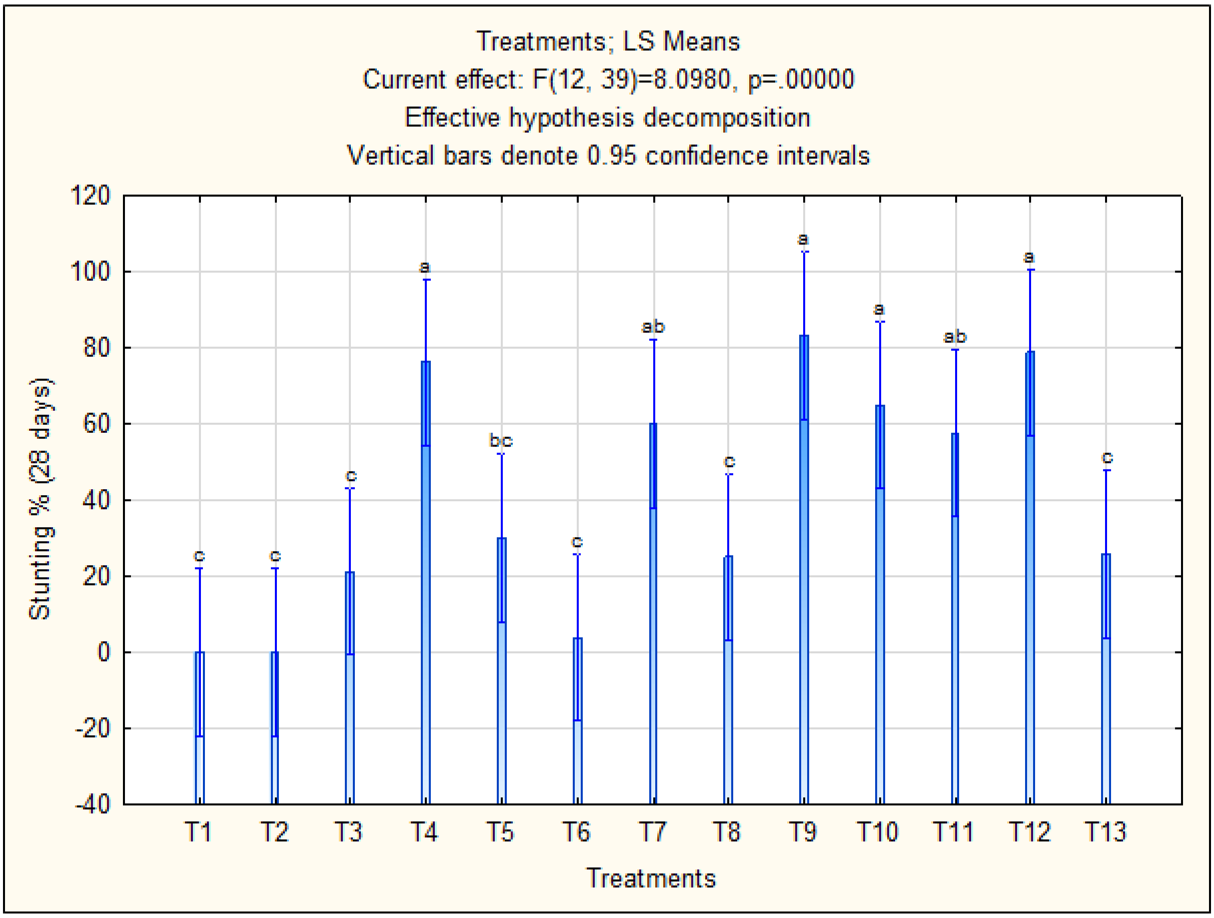


Figure 5.9: Effects of clethodim in combination with various adjuvants on stunting of volunteer maize at 28 DAA, Morgenzon. (See Table 5.2 for treatment descriptions). Different letters at data points indicate significant differences between treatments at p = 0.05.

5.3.1.2.3 Mortality

Significant differences were once again observed between treatments (p<0.05). Treatments 4, 9 and 12 achieved 100% mortality and all contains Destinaire™ either solo or in combination with other adjuvants (Figure 5.10). Interlock™ (Treatment 7) also achieved close to 100% mortality.

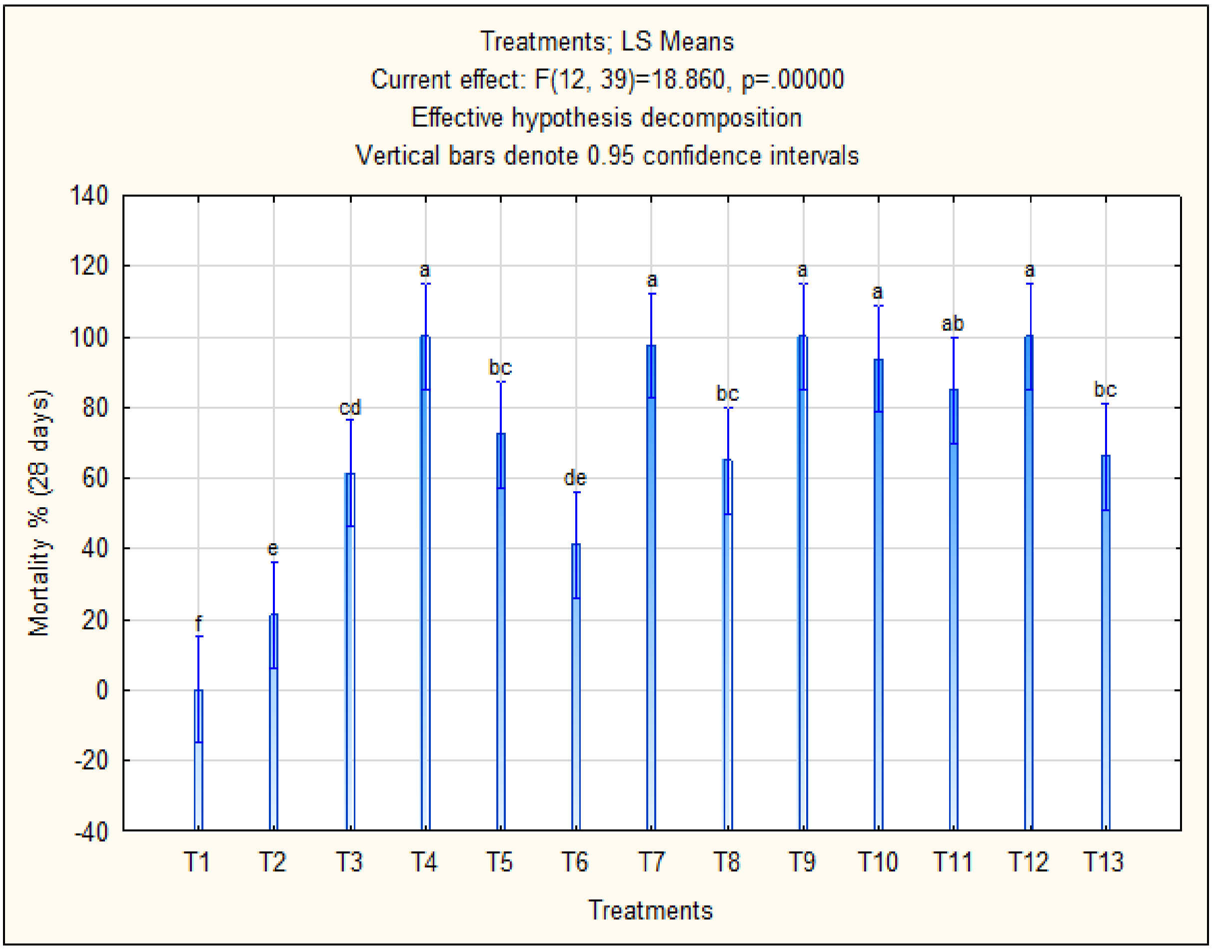


Figure 5.10: Effects of clethodim in combination with various adjuvants on percentage mortality of volunteer maize plants at 28 DAA, Morgenzon. (See Table 5.2 for treatment descriptions). Different letters at data points indicate significant differences between treatments at p = 0.05.

5.3.2 Nelspruit

The trial site at Nelspruit was subjected to a deposition and efficacy trial and the results from that trial site will be presented here.

5.3.2.1 Deposition

The necrosis, stunting and mortality data from 28 days after application (DAA) was used to investigate the impact of different water volumes on the efficacy of herbicide-adjuvant mixtures. Necrosis, stunting, mortality and coverage results will be presented and discussed separately.

5.3.2.1.1 Necrosis

A significant interaction between treatments and water volumes were observed (p<0.05). A general trend was observed that a higher water volume leads to an increase in necrosis (Figure 5.11). A further significant observation to be made is that Destinaire™ solo proved very effective in causing volunteer maize injury. At 150, 200 and 300 L ha^{-1} Destinaire™ combined with clethodim (Treatment 4) provided 97.5% necrosis at 28 DAA (Figure 5.11).

The 80% necrosis threshold analysis revealed that Destinaire™ solo with clethodim reached the threshold in the shortest amount of time at 14 days (Figure 5.12). This is a valuable observation to make because the time taken to reach the 80% threshold is the same for 150, 200 and 300 L ha^{-1}.

5.3.2.1.2 Stunting

Water volume and treatments caused a significant interaction ($p<0.05$). At 28 DAA the 200 and 300 L ha^{-1} treatments caused the highest percentage stunting as is evident in Figure 5.13. Destinaire™ solo with clethodim (Treatment 4) once again proved most effective in causing volunteer maize injury at 150, 200 and 300 L ha^{-1} (Figure 5.13). Treatment 8, Direct + Class Act NG™ + clethodim also provided efficient stunting of the volunteer maize at 150, 200 and 300 L ha^{-1}. The 80% stunting threshold showed that 100 L ha^{-1} reached the threshold in the shortest amount of time (Figure 5.14). This may be due to a shortage in overall stunting at 100 L ha^{-1} (Figure 5.13).

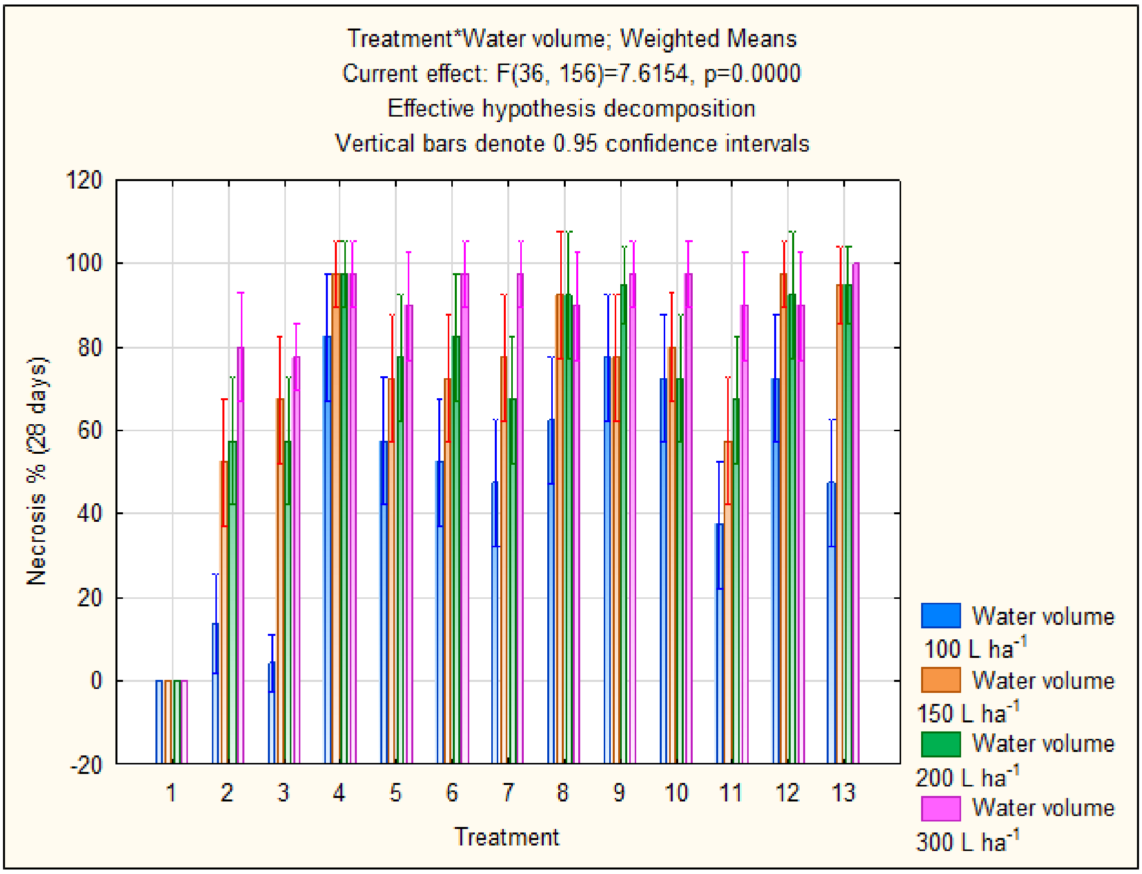


Figure 5.11: Effects of clethodim in combination with various adjuvants on necrosis of volunteer maize plants at 28 DAA, Nelspruit. (See Table 5.2 for treatment descriptions).

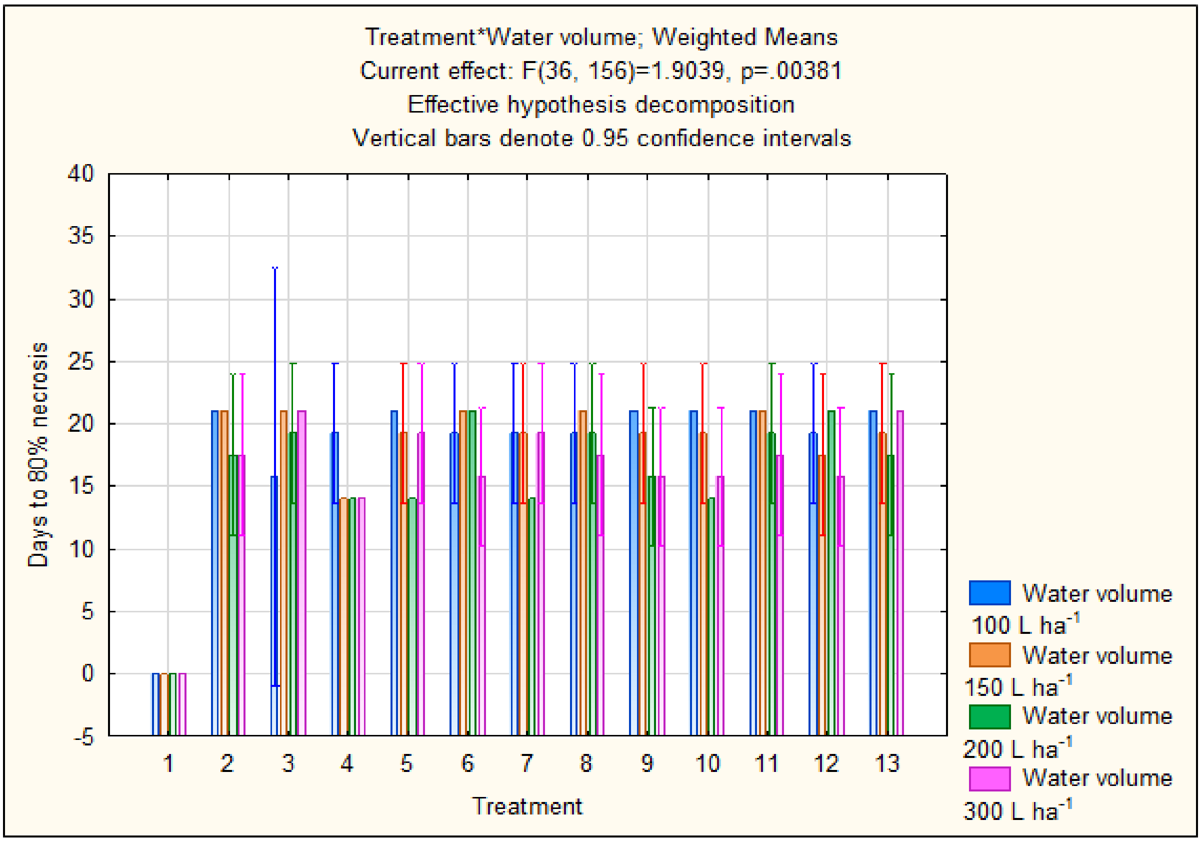


Figure 5.12: The effects of different adjuvants added to clethodim on days to 80% necrosis threshold of volunteer maize plants at 28 DAA, Nelspruit. (See Table 5.2 for treatment descriptions).

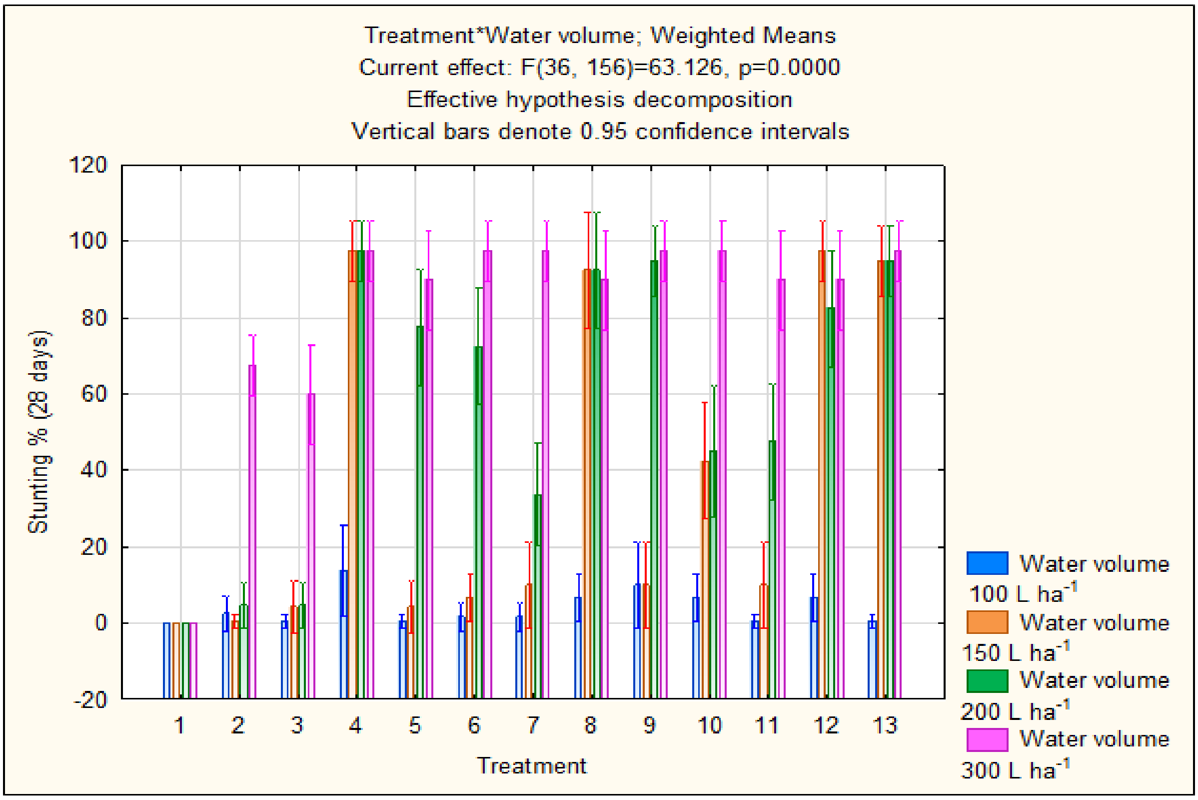


Figure 5.13: Effects of clethodim in combination with various adjuvants on stunting of volunteer maize plants at 28 DAA, Nelspruit. (See Table 5.2 for treatment descriptions).

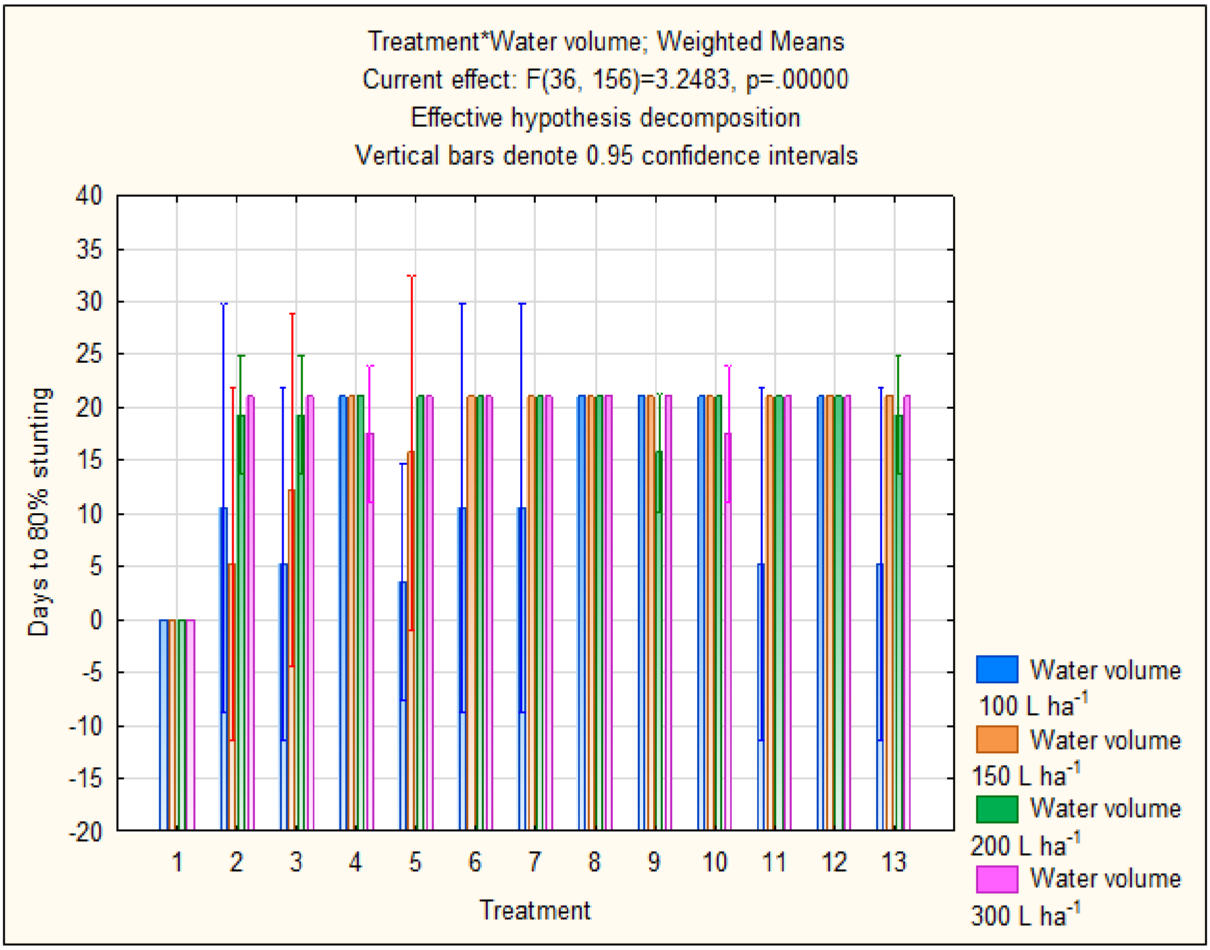


Figure 5.14: The effects of different adjuvants added to clethodim on days to 80% stunting threshold of volunteer maize plants at 28 DAA, Nelspruit. (See Table 5.2 for treatment descriptions).

5.3.2.1.3 Mortality

In terms of the mortality caused by clethodim and various adjuvants on glyphosate-resistant volunteer maize, a significant interaction ($p<0.05$) was observed between water volumes and treatments. A general trend is visible that an increase in water volume produced an increase in mortality (Figure 5.15). At 300 L ha^{-1} clethodim solo (Treatment 2) produced almost 100% mortality which is the first time that a single herbicide performed on par with the adjuvant mixtures at the same water volume (Figure 5.15). Destinaire™ solo with clethodim (Treatment 4) produced high mortality rates (>95%) at all the water volumes (Figure 5.15).

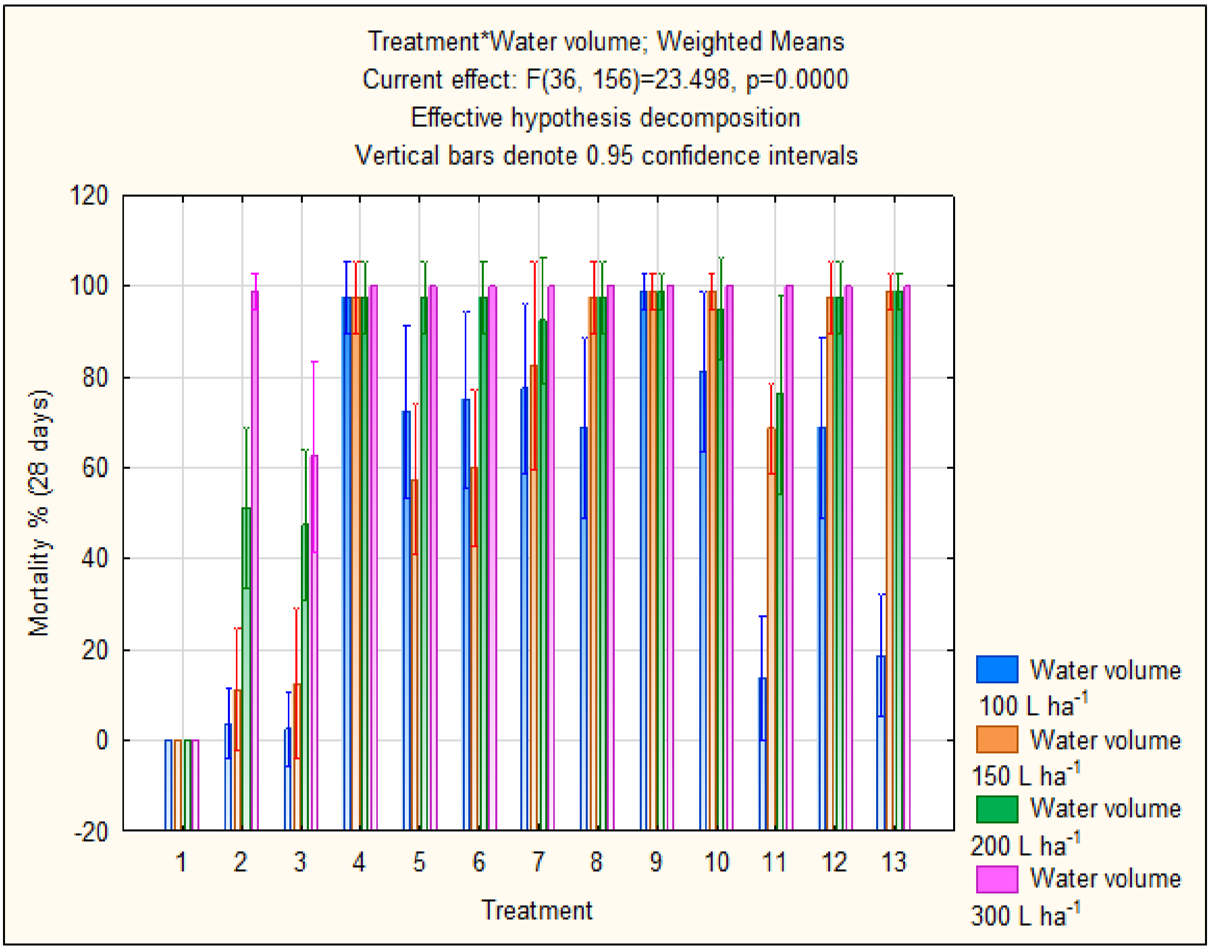


Figure 5.15*:* Effects of clethodim in combination with various adjuvants on percentage mortality of volunteer maize plants at 28 DAA, Nelspruit. (See Table 5.2 for treatment descriptions).

The time taken to reach the 80% threshold showed that 300 L ha^{-1} reached the threshold in the shortest amount of time, between 14 and 19 days. Treatment 2 (clethodim solo) reached the threshold at ± 19 days which was the slowest of any of the treatments at 300 L ha^{-1} (Figure 5.16).

With a decrease in water volume, the time to reach the 80% threshold increased. This is a valuable observation because mortality of volunteer maize plants was observed throughout the water volume spectrum (Figure 5.16).

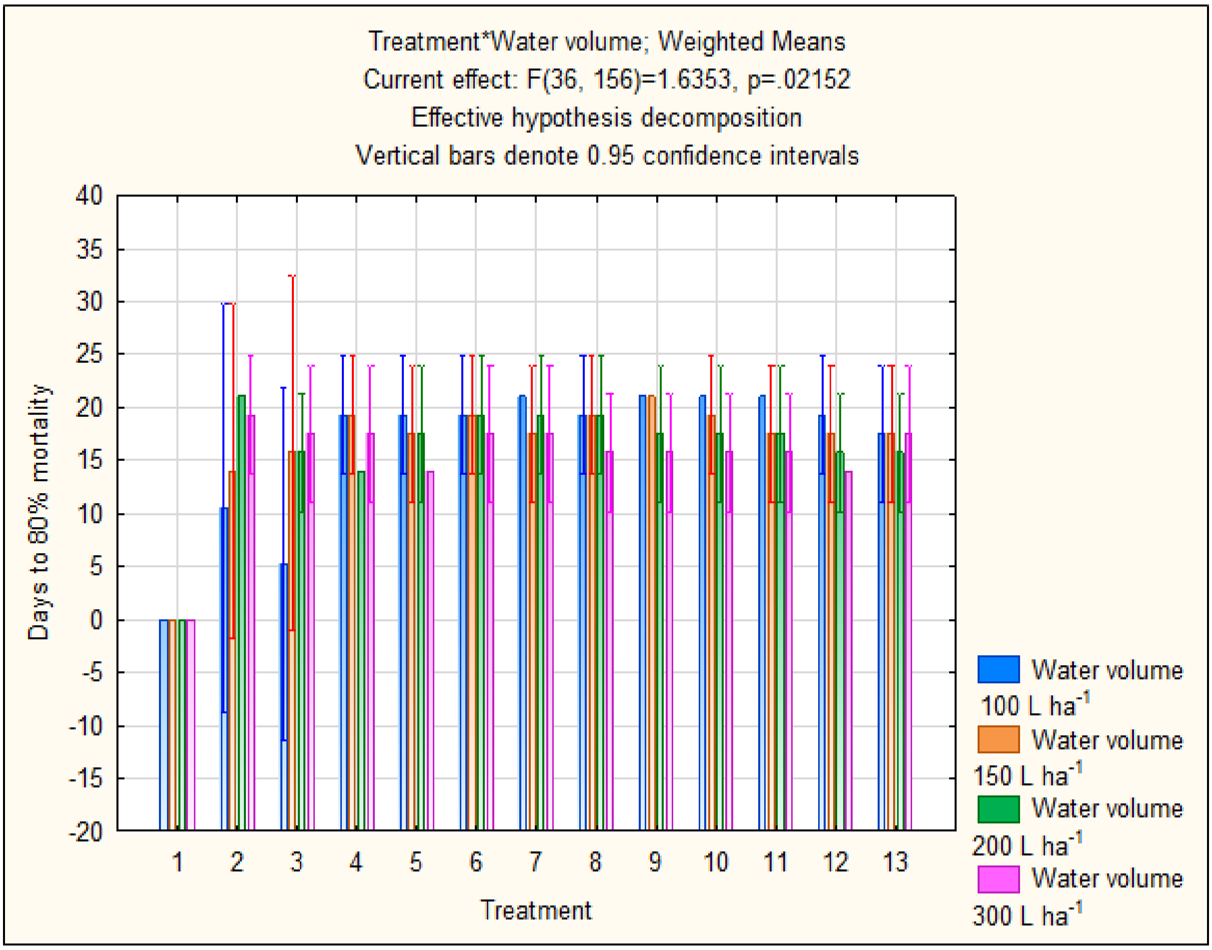


Figure 5.16: The effects of different adjuvants added to clethodim on days to 80% mortality threshold of volunteer maize plants at 28 DAA, Morgenzon. (See Table 5.2 for treatment descriptions).

5.3.2.1.4 Coverage

Water volumes and treatments caused a significant interaction (p<0.05) when percentage coverage that the herbicide-adjuvant mixtures provide is analysed. The expected trend of an increase in coverage with an increase in water volume did not occur (Figure 5.17).

The highest water volume of 300 L ha^{-1} provided the highest percentage coverage as was expected, but 200 L ha^{-1} did not follow the expected trend in providing the second highest percentage cover (Figure 5.17). A lower water volume of 150 L ha^{-1} provided the second highest coverage percentage followed by 100 L ha^{-1}. The lowest coverage percentage was provided by a water volume of 200 L ha^{-1} (Figure 5.17). At the prescribed water volume of 200 L ha^{-1} Destinaire™ + clethodim provided numerically the highest percentage cover when compared to the other treatments at the same water volume (Figure 5.17).

When applied at 150 L ha^{-1} Destinaire™+ Class Act NG™ + clethodim (Treatment 9) caused the highest percentage coverage overall followed by the solo application of clethodim at 300 L ha^{-1} (Figure 5.17). The overall inconsistent performance by any of the treatments does not lend itself to making any further worthwhile observations.

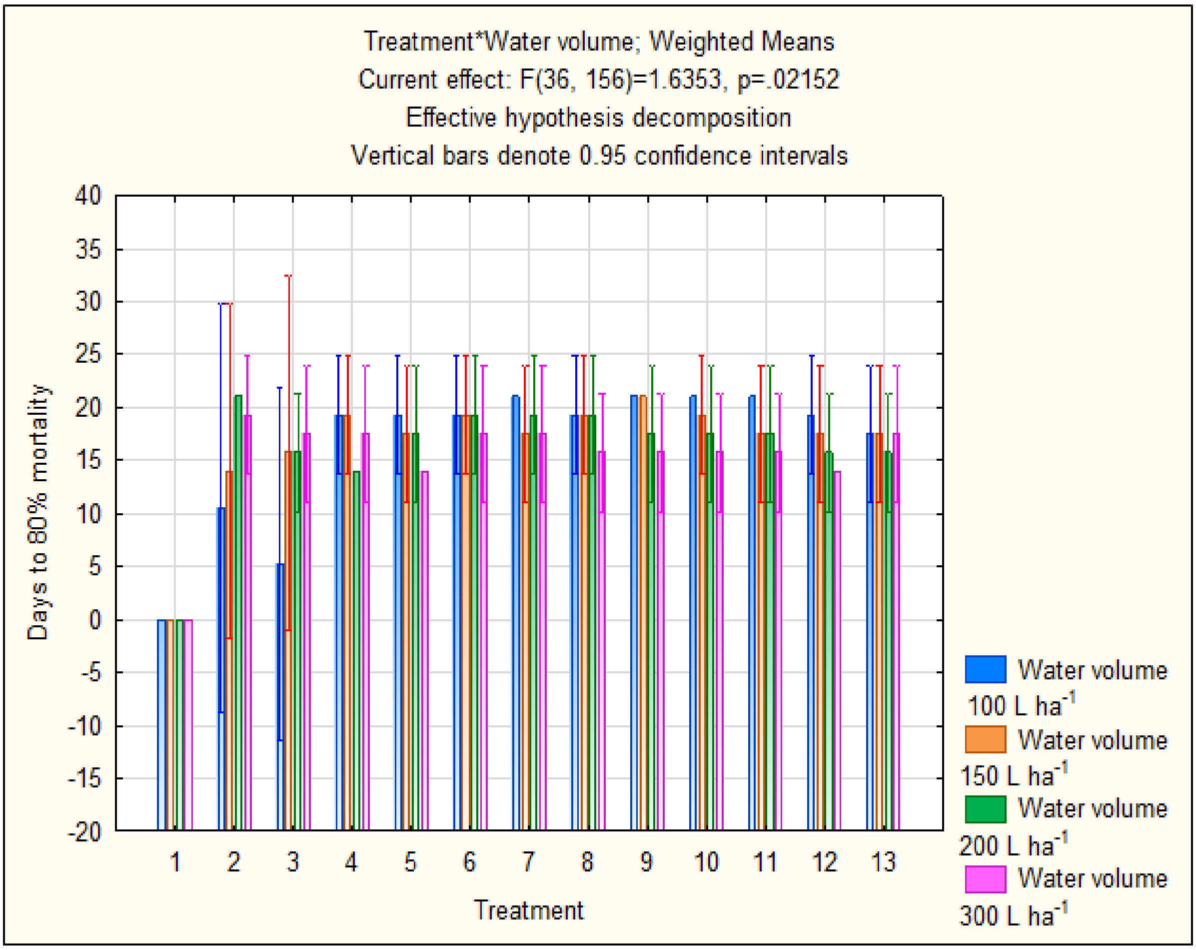


Figure 5.17: The effect of water volume on coverage of the herbicide-adjuvant mixtures on volunteer maize plants, Nelspruit. (See Table 5.2 for treatment descriptions).

5.3.2.2 Efficacy

The necrosis, stunting and mortality data at 28 DAA was used to determine the efficacy of herbicide-adjuvant mixtures to control glyphosate-resistant volunteer maize.

5.3.2.2.1 Necrosis

A univariate test for significance revealed that there are significant differences between treatments ($p<0.05$). Destinaire™ solo + clethodim (Treatment 4) produced numerically the highest percentage necrosis of all the solo adjuvant mixtures. Destinaire™ + Class Act NG™ + clethodim (Treatment 9) and Destinaire™ + Interlock™ + clethodim (Treatment 12) produced the highest necrosis percentages at 85% and 82.5% respectively (Figure 5.18). The solo applications of Direct, Summit Super and Interlock™ with clethodim (Treatments 3, 5 and 7) as well as Treatments 10 and 11 resulted in significantly ($p < 0.05$) less necrosis than the rest of the treatments.

5.3.2.2.2 Stunting

Treatment 4, Destinaire™ + clethodim, provided numerically the highest stunting percentage of the solo adjuvant mixtures at 38.75%. Treatment 9, Destinaire™ + Class Act NG™ + clethodim caused the highest overall stunting percentage with 57.5% followed by treatment 12 with 48.75% (Figure 5.19) These treatments together with Treatments 7 and 13 caused significantly (p<0.05) more stunting than the other treatments.

5.3.2.2.3 Mortality

At 28 DAA Treatments 4, 9, 12 and 13 all provided >80% mortality (Figure 5.20). Treatment 4 caused the highest mortality of the solo adjuvant mixtures and consisted of Destinaire™ + clethodim. Treatment 9 provided the highest overall mortality followed by Treatment 12 (Figure 5.20). Treatments 5, 7, 8 and 10 also resulted in significantly (p<0.05) higher mortality than the rest of the treatments.

Treatments 4, 9 and 12 all contained Destinaire™ which emphasizes the role this adjuvant plays in improving the efficacy of clethodim to control glyphosate-resistant volunteer maize (See Table 5.2 for treatments descriptions).

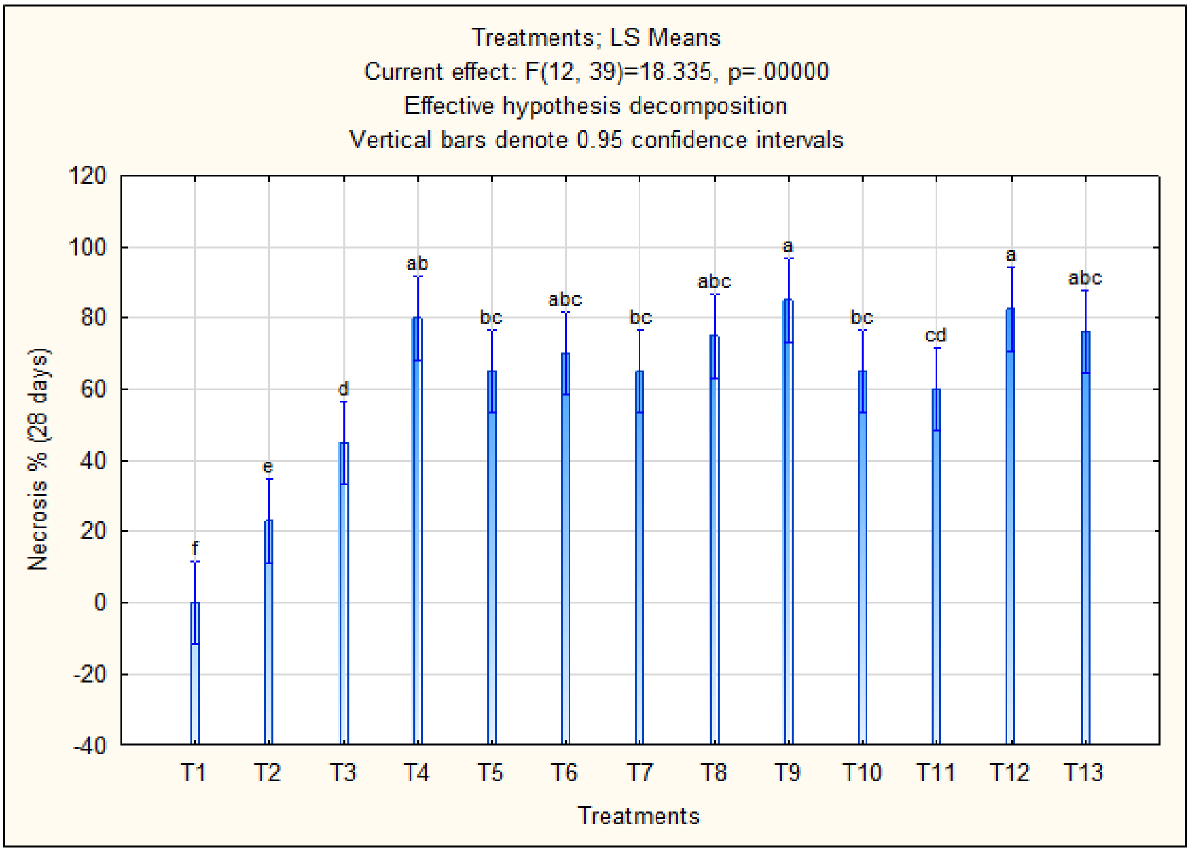


Figure 5.18: Effects of clethodim in combination with various adjuvants on necrosis of volunteer maize plants at 28 DAA, Nelspruit. (See Table 5.2 for treatment descriptions). Different letters at data points indicate significant differences between treatments at p = 0.05.

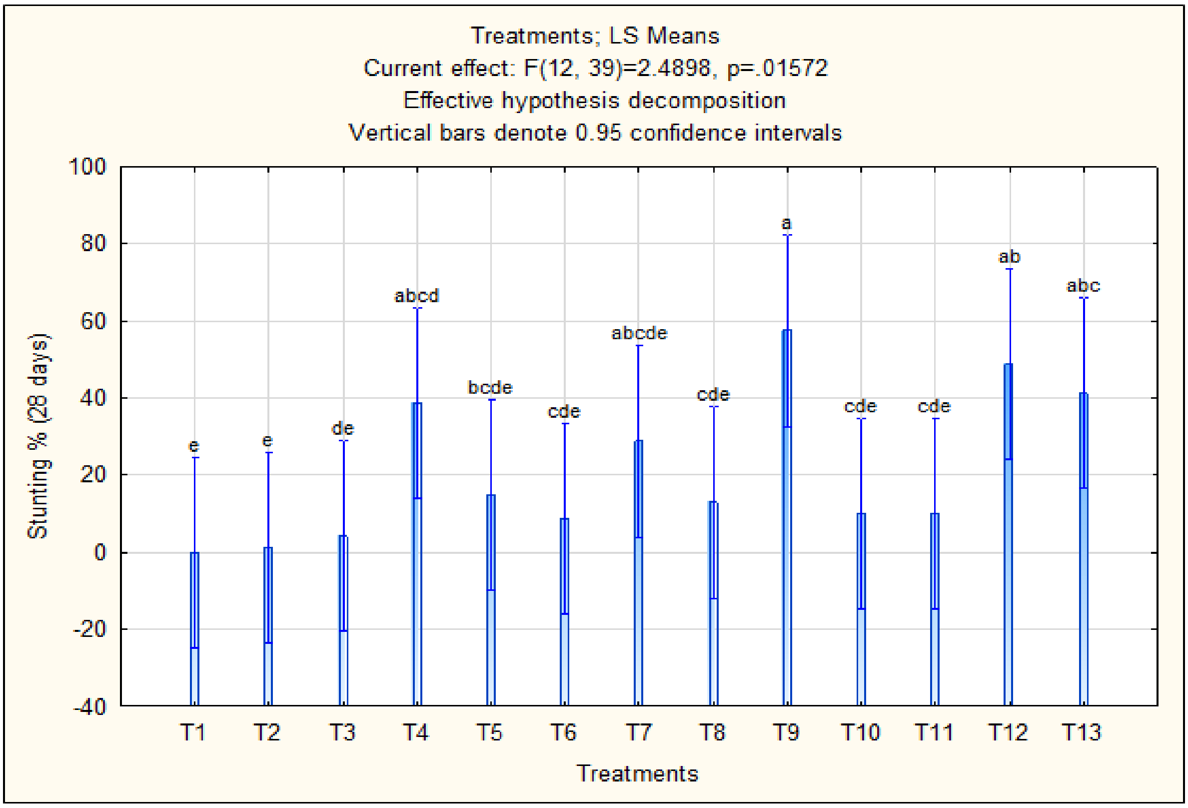


Figure 5.19: Effects of clethodim in combination with various adjuvants on stunting of volunteer maize plants at 28 DAA, Nelspruit. (See Table 5.2 for treatment descriptions). Different letters at data points indicate significant differences between treatments at p = 0.05.

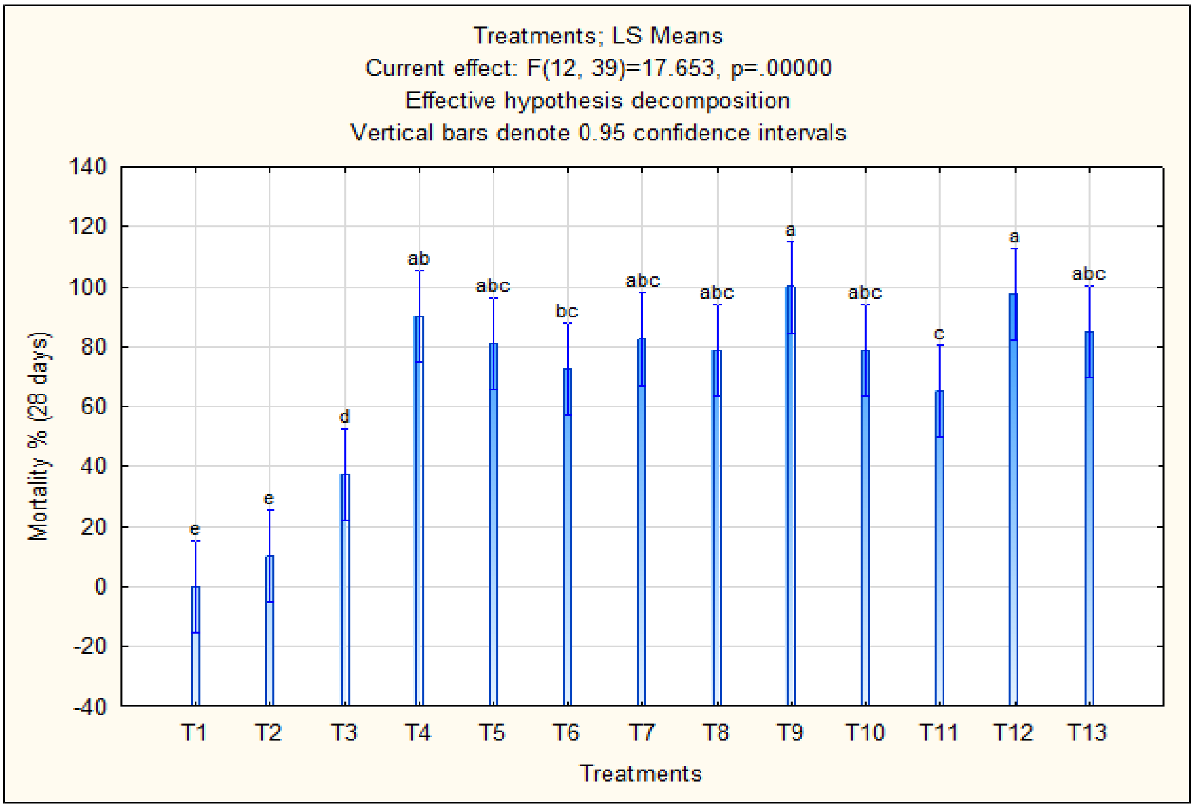


Figure 5.20: Effects of clethodim in combination with various adjuvants on mortality of volunteer maize plants at 28 DAA, Nelspruit. (See Table 5.2 for treatment descriptions). Different letters at data points indicate significant differences between treatments at p = 0.05.

5.4 Discussion

The data from the deposition and efficacy trial at both trial sites were very similar and thus a general discussion, integrating data from both trial sites is presented.

Two general trends arose and duplicated themselves at both trial sites. The first of the trends are that an increase in water volume led to an increase in the efficacy of clethodim to control volunteer maize.

When one considers necrosis, it is clear in Figure 5.1 and Figure 5.11 that an increase in water volume led to an increase in necrosis severity of volunteer plants. The same trend arises when stunting (Figure 5.3 and Figure 5.13) and mortality (Figure 5.5 and Figure 5.15) are considered. The increase in efficacy to control glyphosate-resistant volunteer maize with an increase in water volume was expected (Ramsdale and Messersmith 2001).

The second trend was that Destinaire™ proved to be the adjuvant that increased the efficacy of clethodim the most (Calpepper et al. 1999). Destinaire was responsible, as a solo adjuvant mixture and in combination with other adjuvants, for the highest percentages of necrosis, stunting and mortality of volunteer maize when combined with clethodim. During the necrosis evaluations of the deposition trial and efficacy trial at both trial sites, Destinaire™ was responsible for the highest necrosis percentages both as a solo adjuvant and in combination with other adjuvants (Figures 5.1, 5.8, 5.11 and 5.18).

The stunting evaluations revealed the same trend, that Destinaire™ facilitates the highest volunteer maize injury severity when mixed with clethodim. Destinaire™ was the adjuvant with the highest stunting percentages as a solo adjuvant mixture and in combination with other adjuvants (Figures 5.3, 5.9, 5.13, 5.19). Special mention has to be made for Destinaire™ solo with clethodim (Treatment 4) which provided 50% stunting at 100 L ha^{-1} during the deposition trial in Morgenzon. This equals the highest solo adjuvant stunting percentage at 300 L ha^{-1} and surpassing any other solo adjuvant mixture at any of the other water volumes (Figure 5.3).

Mortality is the most important objective in control of glyphosate-resistant volunteer maize. Destinaire™ once again provided the highest readings. In Morgenzon and Nelspruit Destiniare™ + clethodim achieved ≥90% mortality at all the water volumes (Figures 5.5 and 5.15). At both trial sites Destinaire™ provided the highest mortality rates as a solo adjuvant mixture and in combination with Class Act NG™ (Treatment 9) and Interlock (Treatment 12). This is evident in the deposition and efficacy trials at Morgenzon and Nelspruit (Figures 5.5, 5.10, 5.15, 5.20).

As was expected a higher water volume led to a higher coverage (Legleiter and Johnson 2016). In Morgenzon it was the prescribed water volume of 200 L ha^{-1} that provided the highest overall coverage (Figure 5.7). In Nelspruit it was the highest applied water volume of 300 L ha^{-1} that produced the highest percentage coverage (Figure 5.17).

From the discussion two worthwhile conclusions can be made. The first is that an increase in water volume will increase the efficacy of clethodim and the various adjuvant mixtures. The highest water volume of 300 L ha^{-1} constantly produced the highest percentages when necrosis, stunting, mortality and coverage are considered.

The second conclusion is that Destinaire™ is the adjuvant that compliments clethodim the most effectively and is the adjuvant that I would recommend to apply with clethodim (Jordan et al. 1996). There seems to be no need to add an additional adjuvant to Destinaire™ as this adjuvant performed exceptionally well especially if one considers mortality where Destinaire™ solo with clethodim produced higher than 90% mortality at all the water volumes compared to the 12.5% produced by clethodim in Morgenzon. This statement is true for this study, but in situations where poor water quality is encountered it may be evident that an ammonium sulphate-containing adjuvant will be needed to ensure the efficacy of clethodim (Jordan et al. 1996).

The synergism observed between Destinaire™ and clethodim is due to the way in which clethodim works, the mode of action (MoA). Clethodim is a systemic herbicide, which insinuates that the herbicide has to be able to enter the target weed and disrupt physiological processes of the target weed (Gunsolus and Curran 1999). Clethodim is able to disrupt the physiological processes of the weed species by inhibiting the formation of new cell membranes eventually leading to death (Baumann et al. 2008).

Due to the MoA of clethodim it is imperative that clethodim enters the plant tissue in as a large amount as possible. Destinaire™ facilitates the penetration of herbicides by softening or disrupting the cuticle of plant leaves, thus leading to increased herbicide penetration (Jordan et al. 2011). The ability of Destinaire™ to aid clethodim in penetrating the plant surface leads to an increased efficacy of clethodim to control glyphosate-resistant volunteer maize (Hess 1999).

An adjuvant like Interlock™ did not increase the efficacy of clethodim because it did not aid in the penetration of the plant surface (Prokop and Veverka 2003). Prokop and Veverka (2003) proved that contact herbicides are more affected by deposition agents compared to systemic herbicides which are more influenced by adjuvants which aid in the absorption and penetration of the herbicide.

5.5 References

Baumann PA, Dotray PA, Prostko EP. 2008. Herbicides: how they work and the symptoms they cause. Available at https://agrilifeextension.tamu.edu/library/gardening/herbicides-how-they-work-and-the-symptoms-they-cause/ [accessed 10 September 2019].

Calpepper AS, Jordan DL, York AC, Corbin FT. 1999. Influence of adjuvants and bromoxynil on absorption of clethodim. *Weed technology* 13: 536-541.

Gunsolus JL, Curran WS. 1999. Herbicides mode of action and injury symptoms. Available at http://appliedweeds.cfans.umn.edu/sites/appliedweeds.cfans.umn.edu/files/herbicide_mode_of_action_and_injury_symptoms.pdf [accessed 20 September 2019].

Hess FD. 1999. Surfactants and additives. *Proceedings of the California Weed Science Society* 51: 156-172.

Jordan DL, Vidrine PR, Griffin JL, Reynolds DB. 1996. Influence of adjuvants on efficacy of clethodim. *Weed technology* 10: 738-743.

Jordan T, Johnson B, Nice G. 2011. Adjuvants used with herbicides: factors to consider. *Purdue Extension Weed Science.* Available at https://ag.purdue.edu/btny/weedscience/Pages/default.aspx [accessed 17 September 2019].

Legleiter TR, Johnson WG. 2016. Herbicide coverage in narrow row soybean as influenced by spray nozzle and carrier volume. *Crop Protection* 83: 1-8.

Prokop M, Veverka K. 2003. Influence of droplet spectra on the efficiency of contact and systemic herbicides. *Plant Soil Environment* 49: 75-80.

Ramsdale BK, Messersmith CG. 2001. Nozzle, spray volume, and adjuvants effects on carfentrazone and imazamox efficacy. *Weed technology* 15: 485-491

Chapter 6: Glufosinate-ammonium

6.1 Introduction:

Glufosinate-ammonium is an inhibitor of glutamine synthase herbicide used to control glyphosate-resistant volunteer maize. To determine the influence of adjuvants on the efficacy of glufosinate-ammonium, to control glyphosate-resistant volunteer maize, the herbicide was combined with five different adjuvants in different combinations (Table 6.2).

The herbicide-adjuvant mixtures were then tested in a deposition and efficacy trial to determine the influence of these adjuvants on the efficacy of glufosinate-ammonium to control the volunteer maize. The deposition trial consisted of necrosis, stunting, mortality and coverage data at different water volumes. The efficacy trial consisted of necrosis, stunting and mortality data at a constant water volume.

6.2 Materials and Methods:

The materials and methods as discussed in Chapter 3 is applicable to glufosinate-ammonium. In this chapter the spraying protocol, which illustrates the different treatment combinations, will be presented. This protocol is the same for both the deposition and efficacy trials.

6.2.1 Protocol

As discussed in Chapter 2, five adjuvants were employed to test the impact adjuvants have on the efficacy of herbicides. In Chapter 2 the five adjuvants were identified as a surfactant/oil adjuvant combination, a surfactant/fertilizer combination, a high surfactant oil concentrate (HSOC) methylated seed oil, a liquid AMS/surfactant/humectant combination and a deposition agent.The adjuvants will be discussed by referring to the product names of these adjuvants (Table 6.1). The product Bound 200 SL was used as the active ingredient glufosinate-ammonium.

Table 6.1: Adjuvants and trade names

Adjuvant	Product name
Surfactant/oil adjuvant combination	Direct
A high surfactant oil concentrate (HSOC) methylated seed oil	Destinaire™
A surfactant/fertilizer combination	Summit Super
A liquid AMS/surfactant/humectant combination	Class Act NG™
Deposition agent	Interlock™

The treatment protocol is available in Table 6.2 and illustrates the different glufosinate-ammonium and adjuvant combinations. It is worth mentioning again that the dosage used for glufosinate-ammonium is half the dosage prescribed by the product label, this was done to exaggerate the adjuvant influence on the efficacy of the herbicides.

Table 6.2: Treatment protocol

Treatment	**Product combination**	**Dosage rate:** l/ha; v/v
1	Untreated control (UTC)	
2	Glufosinate-ammonium	3
3	Glufosinate-ammonium+ Direct	3 + 0.1%
4	Glufosinate-ammonium + Destinaire™	3 + 1
5	Glufosinate-ammonium + Summit Super	3 + 0.3%
6	Glufosinate-ammonium + Class Act NG™	3 + 2%
7	Glufosinate-ammonium + Interlock™	3 + 0.3
8	Glufosinate-ammonium + Direct + Class Act NG™	3 + 0.1% + 2%
9	Glufosinate-ammonium + Destinaire™ + Class Act NG™	3 + 1 + 2%
10	Glufosinate-ammonium + Summit Super + Class Act NG™	3 + 0.3% + 2%
11	Glufosinate-ammonium + Summit Super+ Interlock™	3 + 0.1% + 0.3
12	Glufosinate-ammonium + Class Act NG™ + Interlock™	3 + 0.3% + 0.3

The results for glufosinate-ammonium will be discussed under the following headings: Deposition and efficacy. The deposition results are based on the data retrieved from applying the treatments at 100, 150, 200 and 300 L ha^{-1}. Coverage data for the deposition trial was obtained from analyzing the water sensitive paper that was placed in the deposition plots with the purpose of examining the influence that water volume and adjuvants have on the coverage of herbicides. The efficacy results are based on the efficacy trial done where the treatments were applied at 200 L ha^{-1}.

The deposition and efficacy will be discussed separately for each trial site. The Morgenzon trial site will be referred to as Trial site 1 and the Nelspruit trial site will be referred to as Trial site 2.

6.3 Results

6.3.1 Morgenzon

An efficacy and deposition trial were planted at Morgenzon and the results from that trial site will be discussed here.

6.3.1.1 Deposition

The necrosis, stunting and mortality data from 28 days after application (DAA) was used to investigate the impact of different water volumes on the efficacy of herbicide-adjuvant mixtures.

Necrosis, stunting, mortality and coverage results will be presented and discussed separately.

6.3.1.1.1 Necrosis

The 28 DAA analysis revealed that a significant interaction was observed between water volumes and treatments ($p<0.05$). A very obvious trend surfaced where it was observed that an increase in water volume leads to an increase in necrosis severity (Figure 6.1). Treatments 5, 6, 7, 8, 9, 10, 11 and 12 all produced necrosis percentages of 98.75% at 300 L ha^{-1} which confirms the observation that an increase in water volume lead to an increase in necrosis severity (Figure 6.1).

Treatments 6, 8, 9 and 12 produced the highest necrosis levels at 200 L ha^{-1} compared to the remaining treatments but not all of it was statistically significant (Figure 6.1). These treatments all contained Class Act NG™ and specifically Treatment 6 which was Class Act NG™ solo with glufosinate-ammonium. Treatment 12 at all water volumes also performed very well and consisted of Class Act NG™ + Interlock™ + glufosinate-ammonium (Figure 6.1).

The 80% necrosis threshold revealed that 200 and 300 L ha^{-1} reached the threshold the quickest at between 14 and 15 days (Figure 6.2). This was statistically faster than 100 and 150 L ha^{-1} that reached the threshold between 16 and 18 days (Figure 6.2).

6.3.1.1.2 Stunting

Significant interactions were observed between water volumes and treatments ($p<0.05$). The higher water volumes of 200 and 300 L ha^{-1} delivered the highest percentage stunting as is visible in Figure 6.3. Summit Super solo with glufosinate-ammonium (Treatment 5) produced the highest stunting percentages of the solo adjuvant treatments at 150, 200 and 300 L ha^{-1} (Figure 6.3). Class Act NG™ + Destinaire™ + glufosinate-ammonium (Treatment 9) caused the highest stunting severity of all the treatments at 150, 200 and 300 L ha^{-1} (Figure 6.3).

The 80% stunting threshold analysis revealed that when the treatments were applied at 100 L ha^{-1} the threshold was reached in a shorter amount of time (Figure 6.4). This may be due to the lower stunting percentages at 100 L ha^{-1} as is visible in Figure 6.3.

6.3.1.1.3 Mortality

Mortality evaluations revealed that significant interactions occurred between treatments and water volumes ($p<0.05$). The general trend that an increase in water volume leads to an increase in volunteer maize injury is valid when mortality is considered as well. The water volume of 300 L ha^{-1} produced the highest mortality rate for all the treatments (Figure 6.5). This was followed by 200 L ha^{-1} which produced the second highest mortality for all the treatments (Figure 6.5).

Class Act NG™ solo with glufosinate-ammonium (Treatment 6) caused the highest mortality rate for the solo adjuvant treatments at 100, 200 and 300 L ha^{-1} (Figure 6.5). When adjuvant combinations are considered Treatment 9, Class Act NG™ + Destinaire™ + glufosinate-ammonium, produced the highest mortality rates for 150, 200 and 300 L ha^{-1}(Figure 6.5).

To determine the rate of mortality the 80% mortality threshold was analysed. The interaction between water volumes and treatments is significant ($p<0.05$). When water volumes are considered no general trends are observed but it would seem that Treatment 7, Interlock™ + glufosinate-ammonium, reached the 80% mortality threshold numerically quicker at all the water volumes (Figure 6.6).

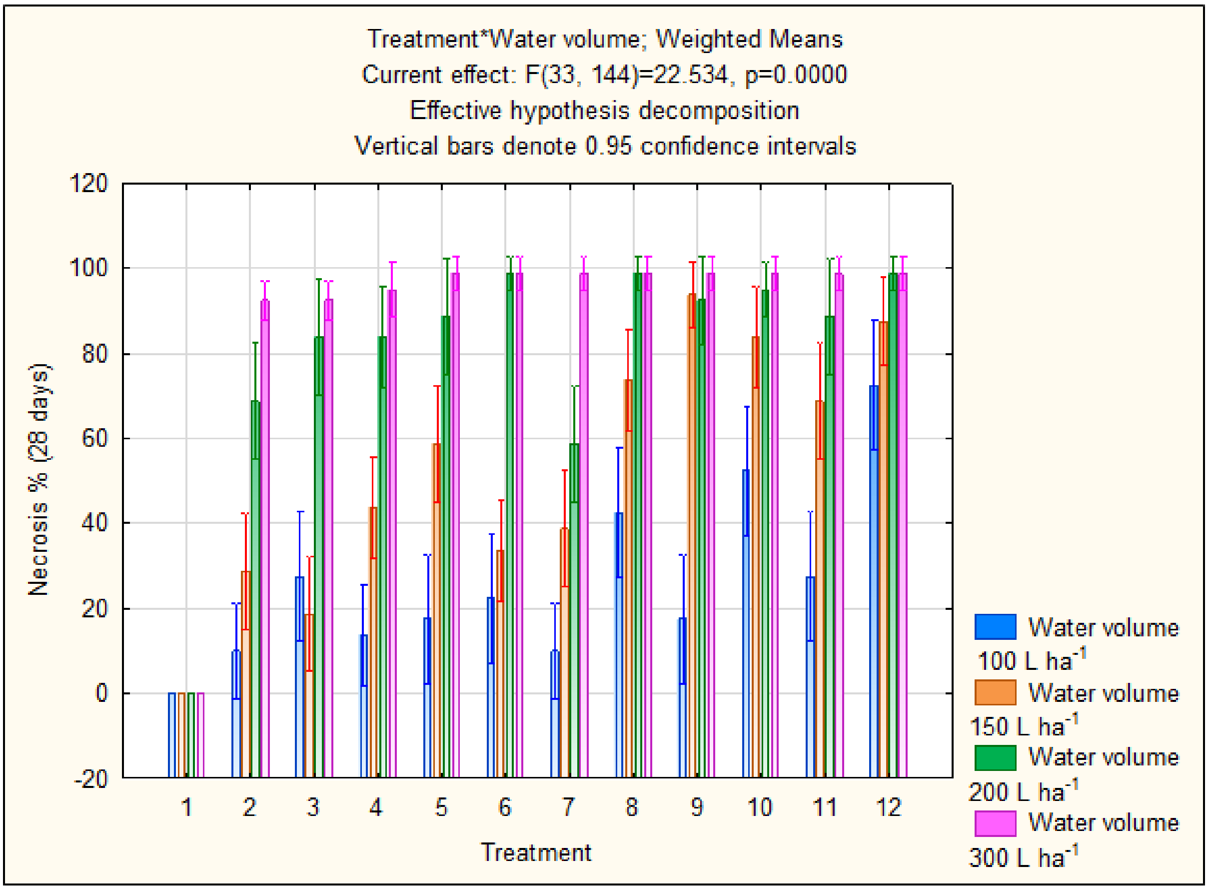


Figure 6.1: Effects of glufosinate-ammonium in combination with various adjuvants on necrosis of volunteer maize plants at 28 DAA, Morgenzon. (See Table 6.2 for treatment descriptions).

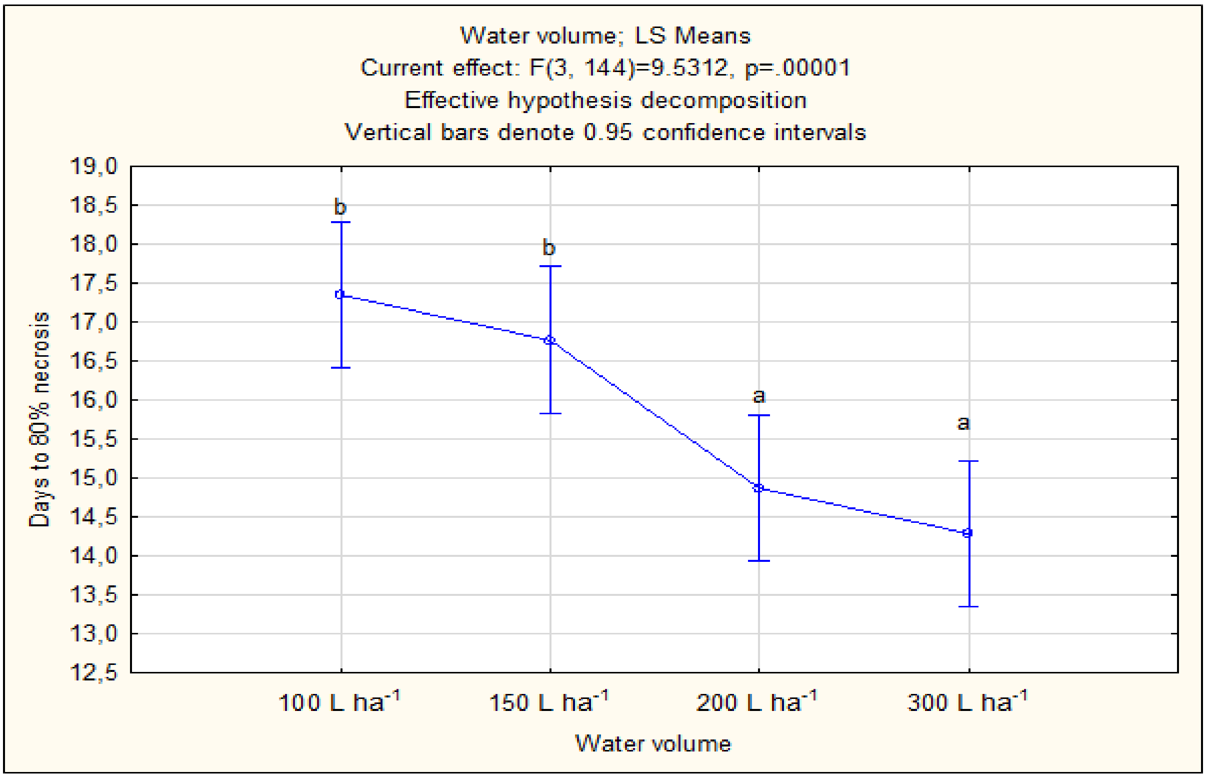


Figure 6.2: The effects of different adjuvants added to glufosinate-ammonium on days to 80% necrosis threshold of volunteer maize plants at 28 DAA, Morgenzon. (See Table 6.2 for treatment descriptions). Different letters at data points indicate significant differences between treatments at p = 0.05.

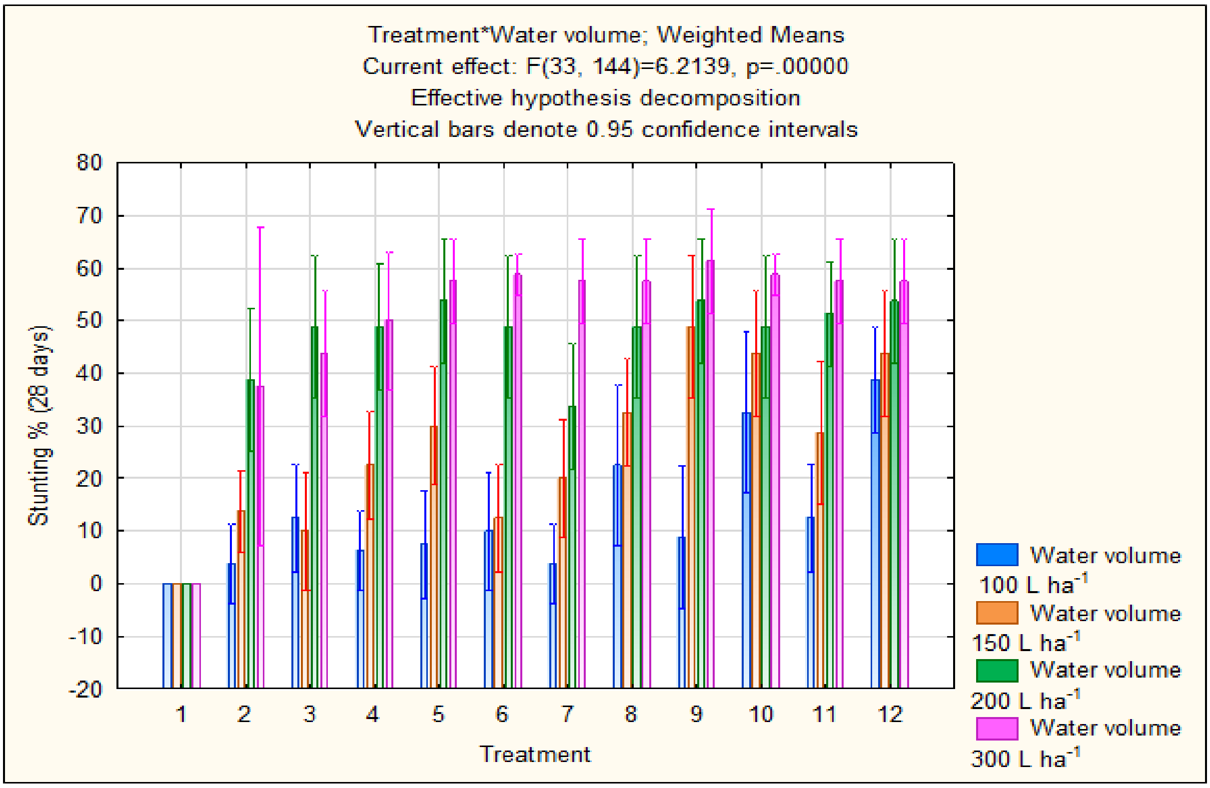


Figure 6.3: Effects of glufosinate-ammonium in combination with various adjuvants on stunting of volunteer maize plants at 28 DAA, Morgenzon. (See Table 6.2 for treatment descriptions).

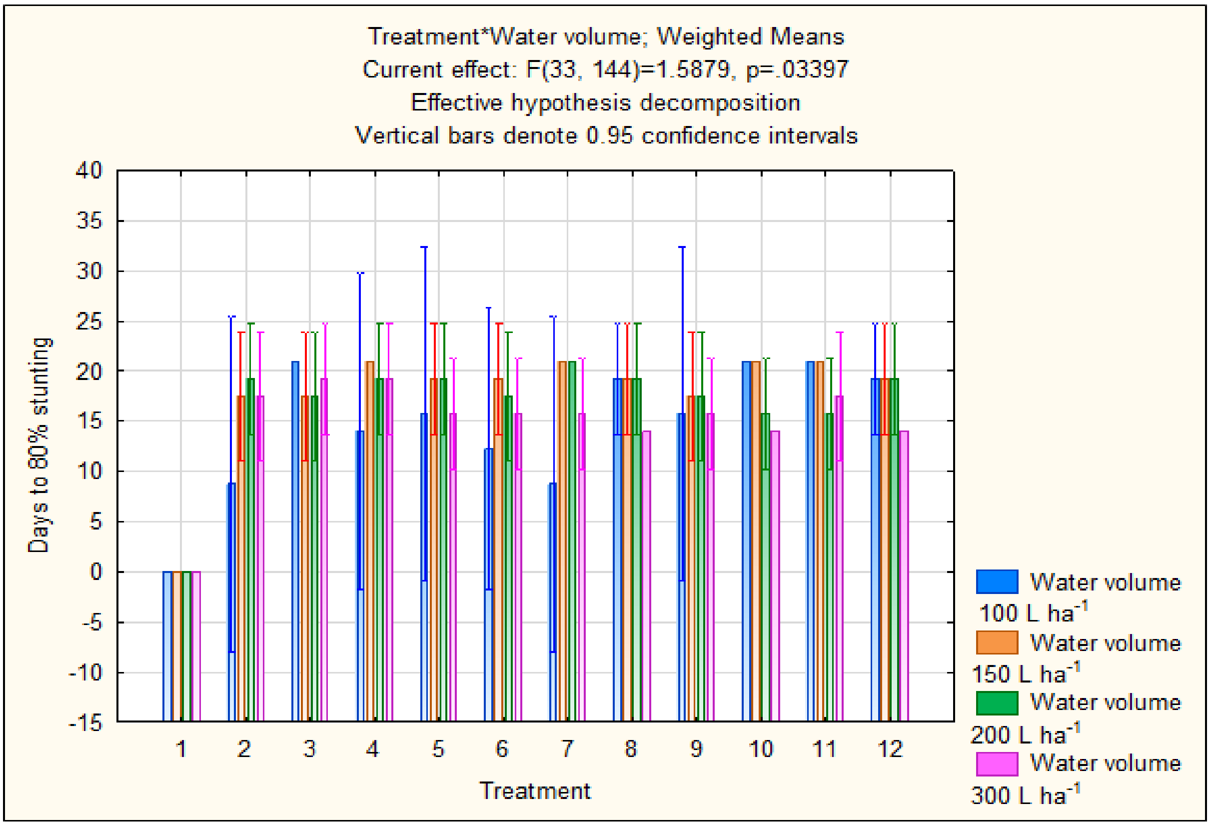


Figure 6.4: The effects of different adjuvants added to glufosinate-ammonium on days to 80% stunting threshold of volunteer maize plants at 28 DAA, Morgenzon. (See Table 6.2 for treatment descriptions).

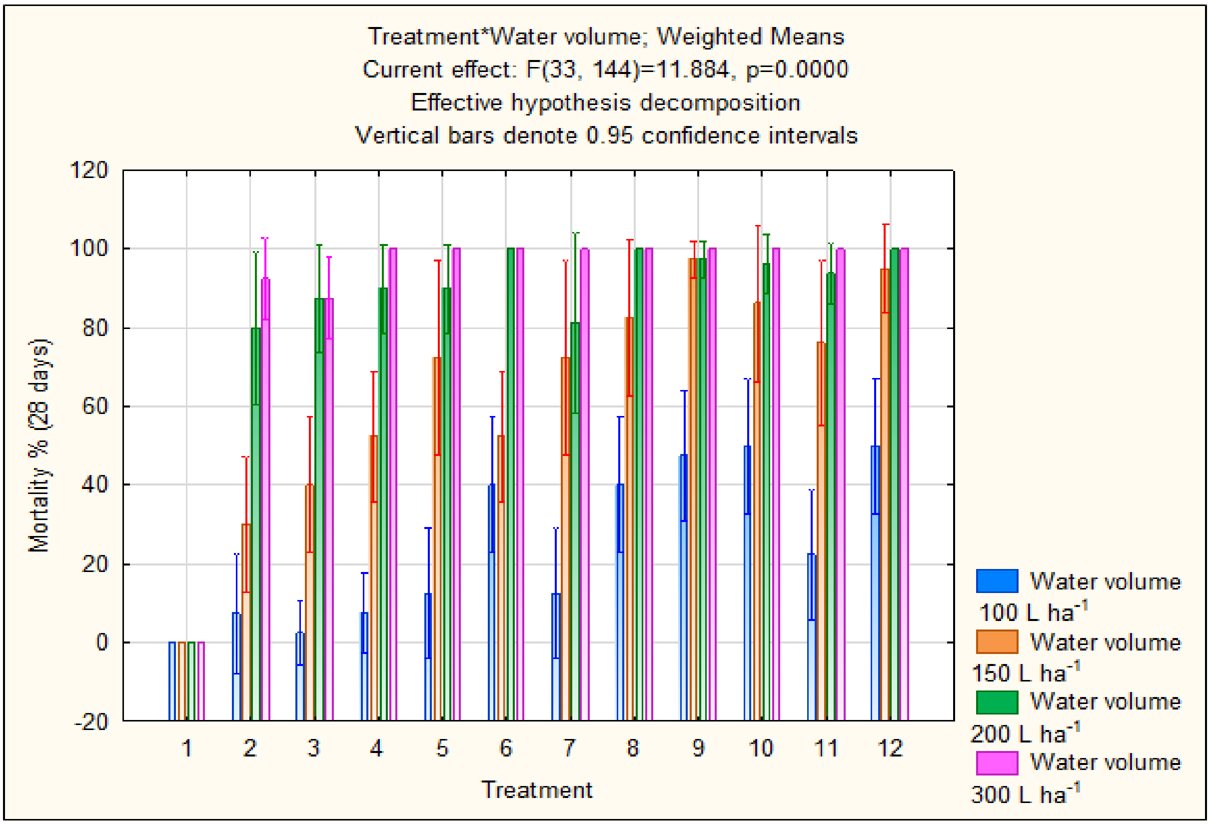


Figure 6.5: Effects of glufosinate-ammonium in combination with various adjuvants on percentage mortality of volunteer maize plants at 28 DAA, Morgenzon. (See Table 6.2 for treatment descriptions).

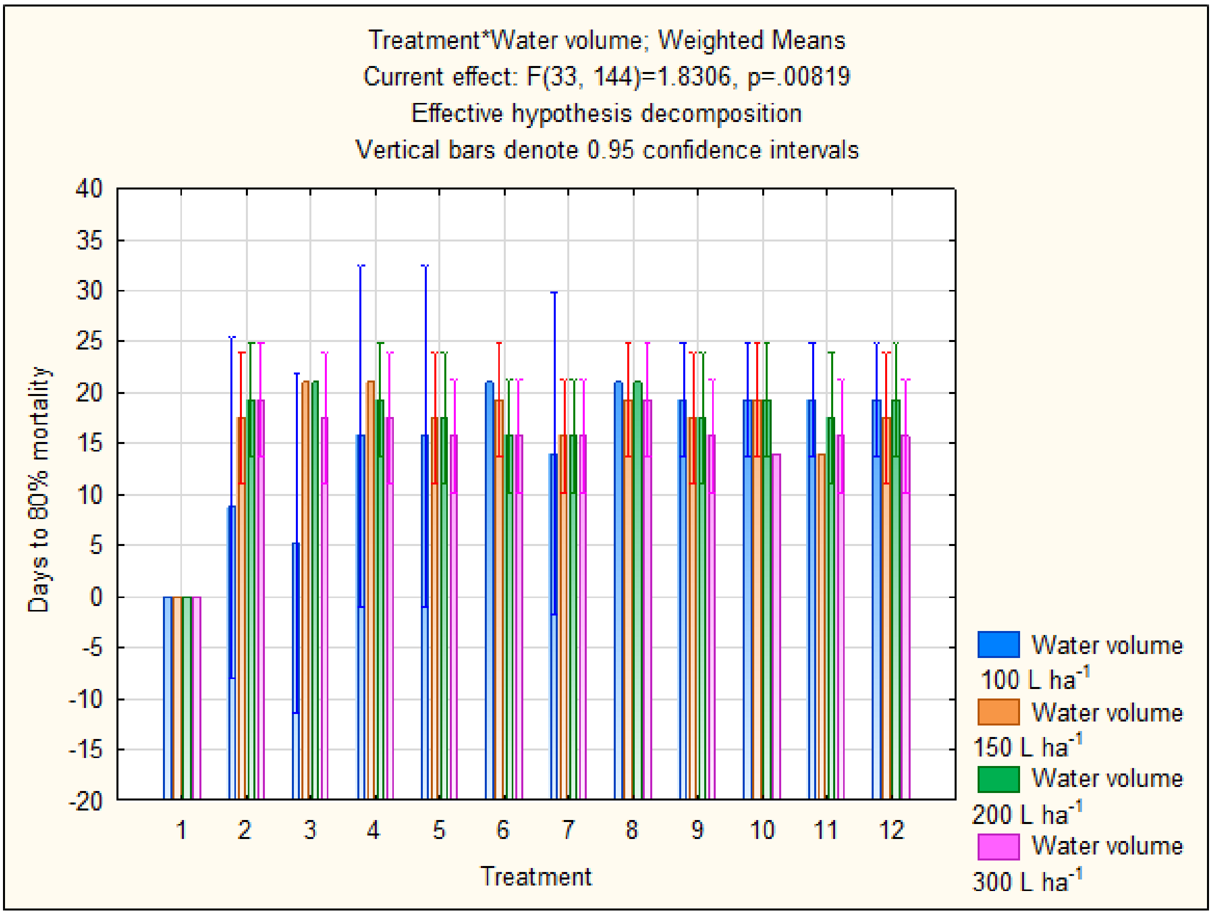


Figure 6.6: The effects of different adjuvants added to glufosinate-ammonium on days to 80% mortality threshold of volunteer maize plants at 28 DAA, Morgenzon. (See Table 6.2 for treatment descriptions).

6.3.1.1.4 Coverage

The percentage coverage analysis caused a significant interaction between water volumes and treatments ($p<0.05$). A water volume of 300 L ha^{-1} generally delivered higher percentage coverage readings (Figure 6.7).

Figure 6.7 revealed that glufosinate-ammonium solo (Treatment 2) delivered the highest overall coverage at 100 and 150 L ha^{-1} without the aid of any an adjuvant. At 300 L ha^{-1} glufosinate-ammonium solo caused the second highest percentage coverage with ±40%, second only to Treatment 12 which contains Class Act NG™ + Interlock™ + glufosinate-ammonium at 57% coverage (Figure 6.7).

Treatment 7, Interlock™ + glufosinate-ammonium, produced coverage peaks at all the water volumes (Figure 6.7). Where glufosinate-ammonium solo produced generally higher coverage percentages when compared to solo adjuvant mixtures it is evident that Interlock™ did aid in the coverage provided (Figure 6.7).

The expected increase in overall coverage with an increase in water volume was not observed and no clear significant differences was observed between water volumes.

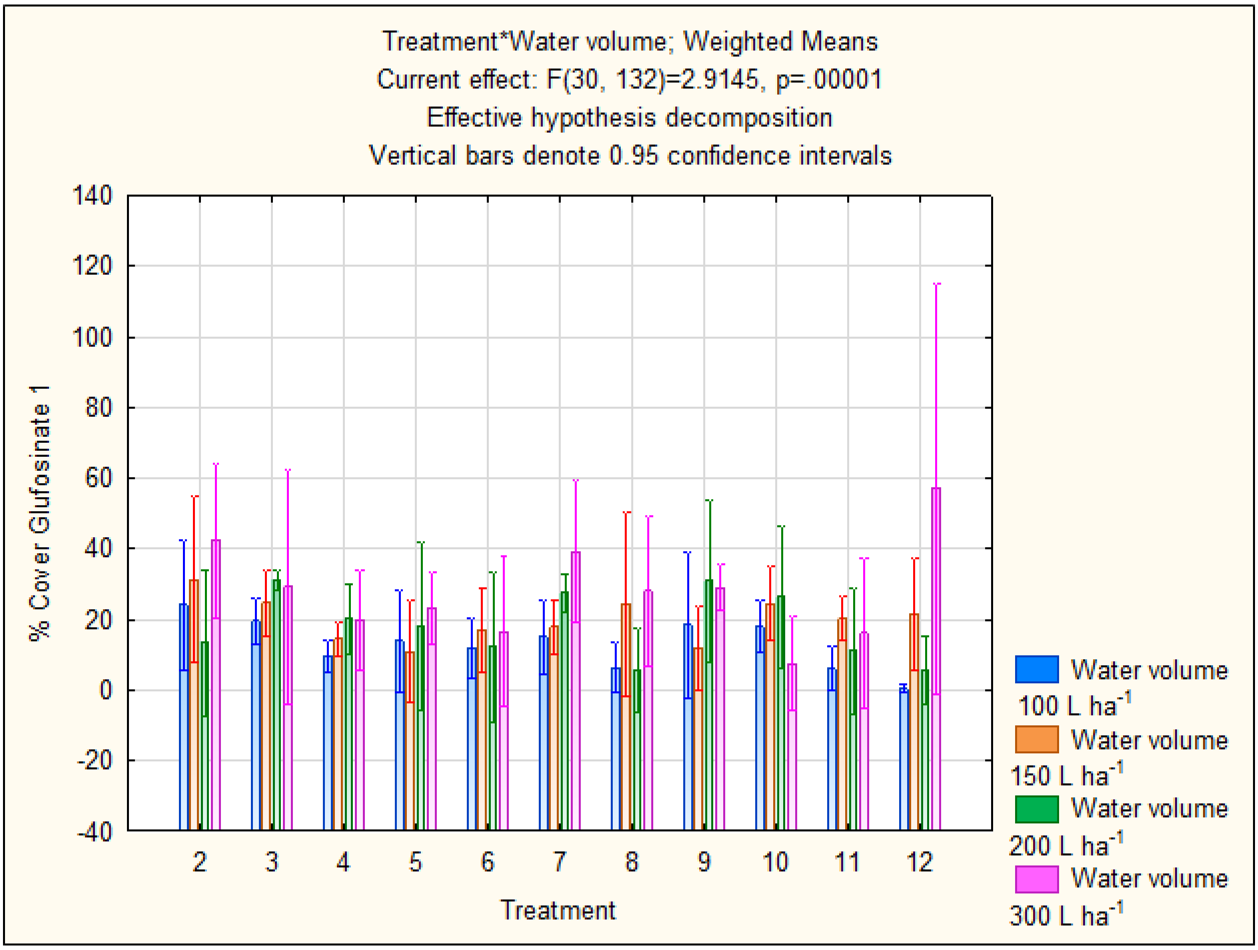


Figure 6.7: The effect of water volume on coverage of the herbicide-adjuvant mixtures on volunteer maize plants, Morgenzon. (See Table 6.2 for treatment descriptions).

6.3.1.2 Efficacy

The necrosis, stunting and mortality data at 28 DAA was used to determine the efficacy of herbicide-adjuvant mixtures to control glyphosate-resistant volunteer maize.

6.3.1.2.1 Necrosis

Treatments 6, 8 and 12 had significantly higher necrosis percentages compared to Treatment 7 (Figure 6.8). The remaining treatments did not differ from one another, but did show statistically more necrosis compared to the untreated control (Figure 6.8).

Treatments 6, 8 and 12 all contained Class Act NG™ (see Table 6.2 for treatment descriptions), which would suggest that Class Act NG™ combined with glufosinate-ammonium produces a synergism that causes extensive volunteer maize injury.

6.3.1.2.2 Stunting

The stunting evaluation caused the same trend where Treatments 6,8 and 12 delivers the highest numerical stunting percentages. Those three treatments also had statistically more stunting than Treatment 4 (Figure 6.9).

6.3.1.2.3 Mortality

When mortality was analysed no significant differences were observed between treatments (Figure 6.10).

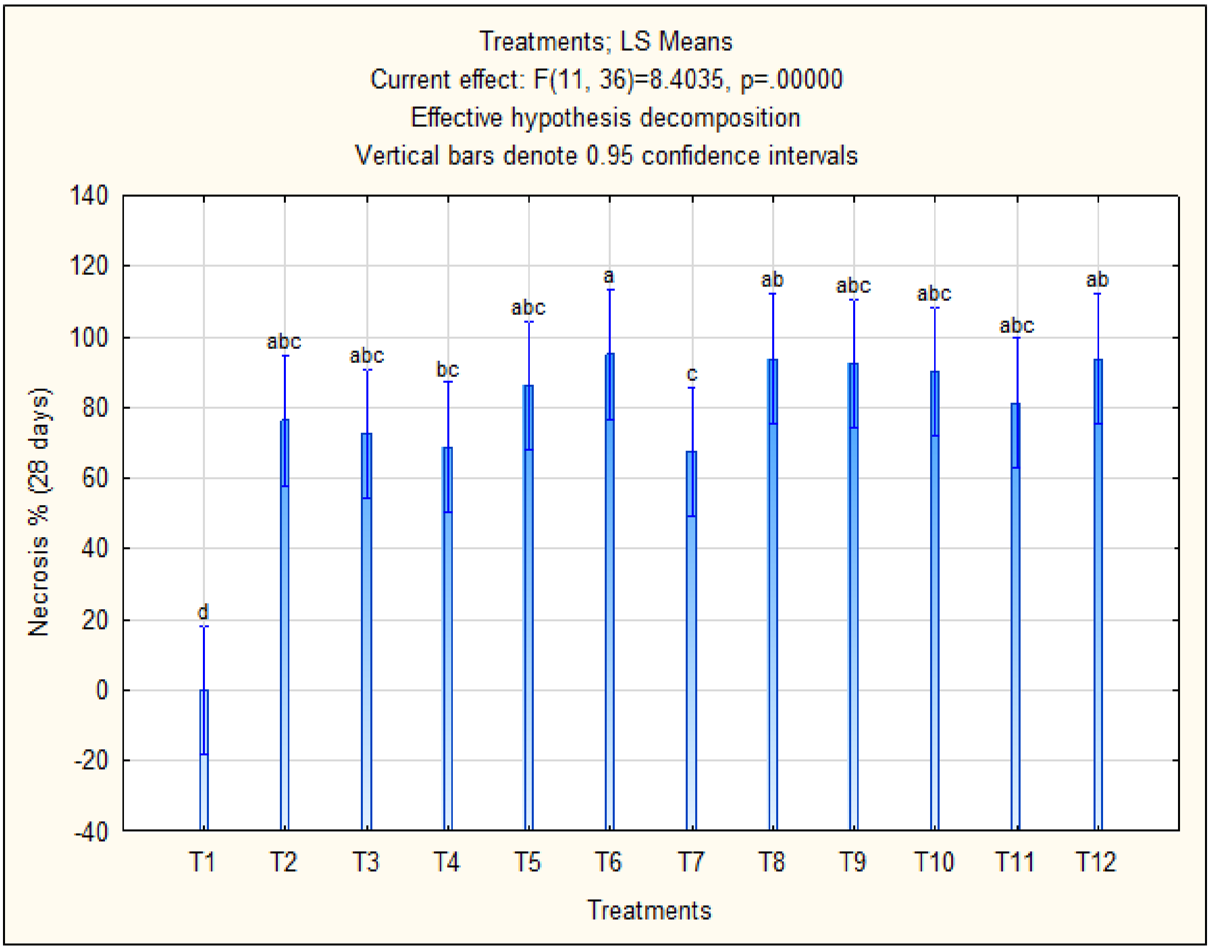


Figure 6.8: Effects of glufosinate-ammonium in combination with various adjuvants on necrosis of volunteer maize plants at 28 DAA, Morgenzon. (See Table 6.2 for treatment descriptions). Different letters at data points indicate significant differences between treatments at p = 0.05.

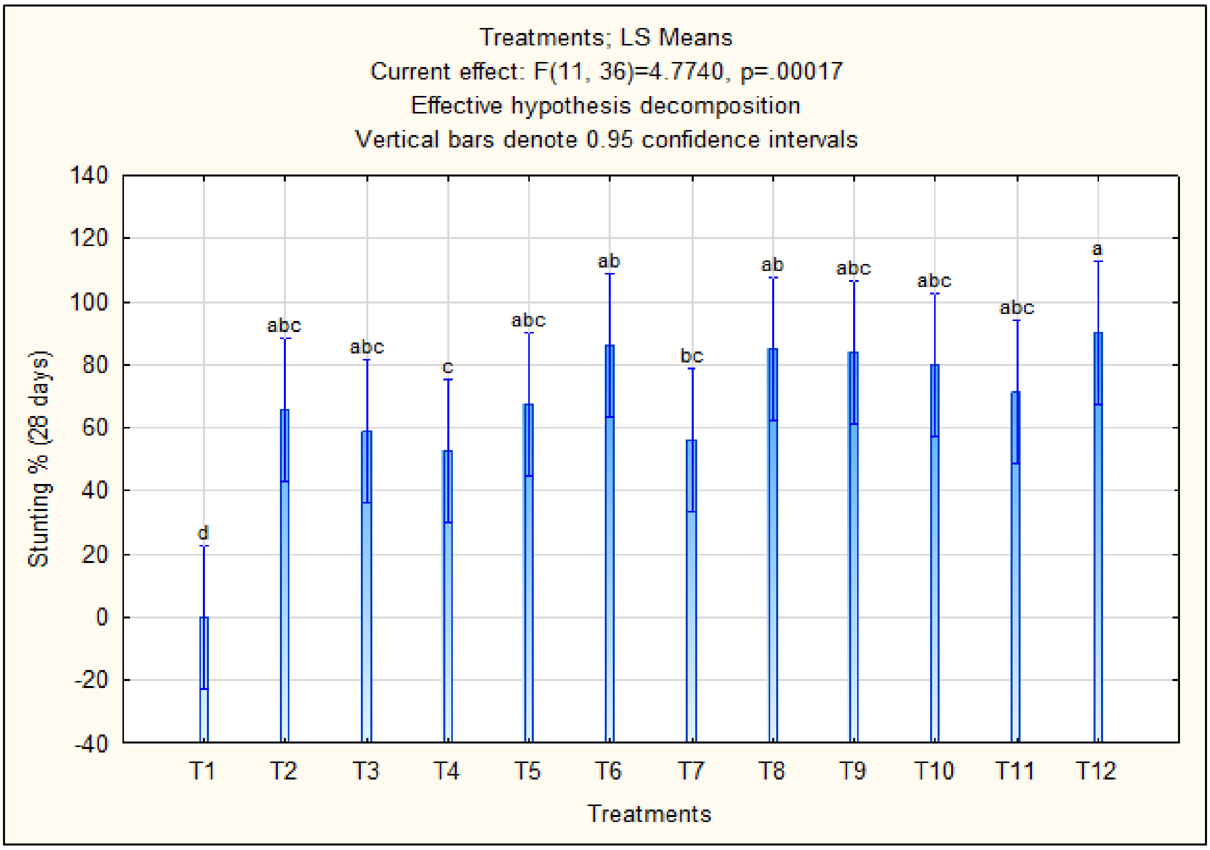


Figure 6.9: Effects of glufosinate-ammonium in combination with various adjuvants on stunting of volunteer maize plants at 28 DAA, Morgenzon. (See Table 6.2 for treatment descriptions). Different letters at data points indicate significant differences between treatments at p = 0.05.

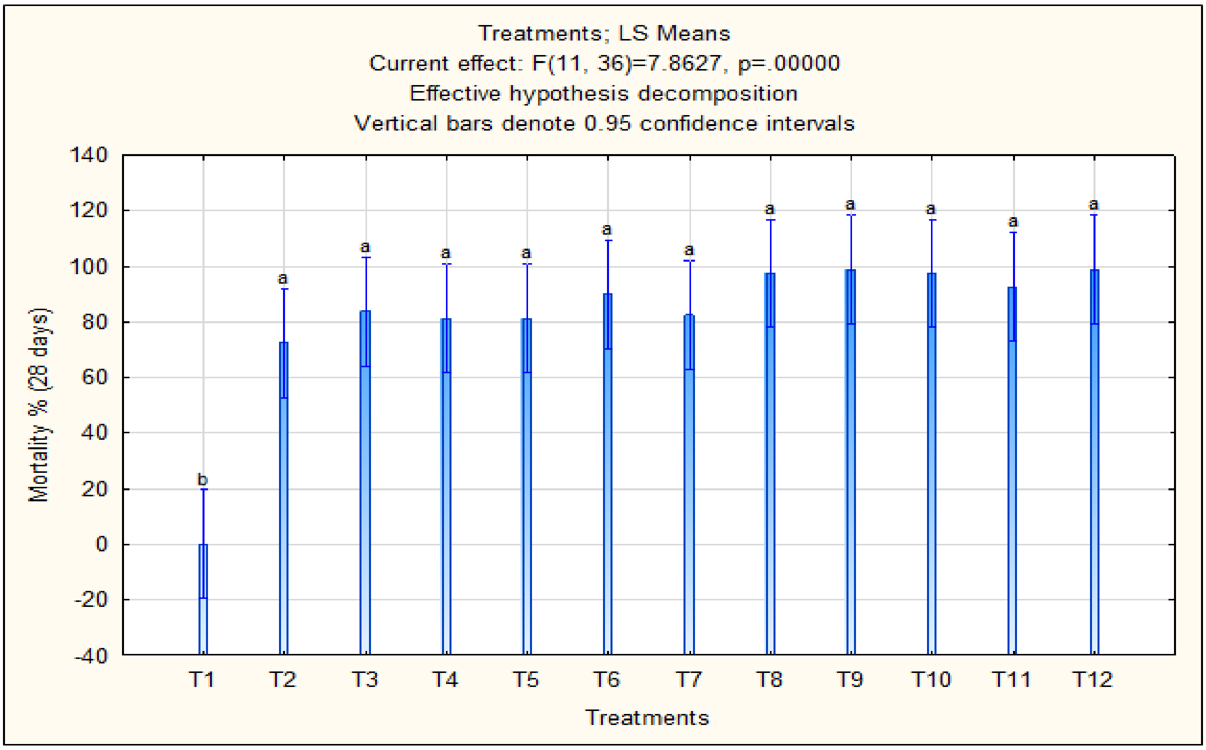


Figure 6.10: Effects of glufosinate-ammonium in combination with various adjuvants on mortality of volunteer maize plants at 28 DAA, Morgenzon. (See Table 6.2 for treatment descriptions). Different letters at data points indicate significant differences between treatments at p = 0.05.

6.3.2 Nelspruit

The trial site at Nelspruit was subjected to a deposition and efficacy trial and the results from that trial site will be presented here.

6.3.2.1 Deposition

The necrosis, stunting and mortality data from 28 days after application (DAA) was used to investigate the impact of different water volumes on the efficacy of herbicide-adjuvant mixtures. Necrosis, stunting, mortality and coverage results will be presented and discussed separately.

6.3.2.1.1 Necrosis

The interaction between water volumes and treatments were investigated and revealed that it was significant ($p<0.05$).

An increase in water volume led to an increase in necrosis (Figure 6.11). The higher water volumes of 200 and 300 L ha^{-1} delivered higher overall necrosis percentages (Figure 6.11). The lower water volumes of 100 and 150 L ha^{-1} resulted in lower overall necrosis percentages but there was no clear trend of one being more efficient than the other.

Treatment 6, Class Act NG™ + glufosinate-ammonium, was responsible for the highest necrosis severity at 200 L ha^{-1} with 97,5%. At 300 L ha^{-1} Treatment 6 was once again part of the group of treatments that delivered the highest, 100%, necrosis severity along with treatments 4, 5, 8, 9, 10, 11 and 12 (Figure 6.11). Treatments 8, 9, 10 and 12 also contain Class Act NG™ in combination with various other adjuvants + glufosinate-ammonium (Table 6.2).

6.3.2.1.2 Stunting

The stunting evaluation resulted in an increase in water volume lead to an increase in stunting severity (Figure 6.12). Class Act NG™ (Treatment 6) once again proved to be a prominent adjuvant both as a solo adjuvant and in combination with other adjuvants.

The 200 and 300 L ha^{-1} water volumes produced the most severe stunting with the higher of the two causing the highest stunting percentage (Figure 6.12). Destinaire™ + glufosinate-ammonium and Class Act NG™ + glufosinate-ammonium were solo adjuvant treatments (treatments 4 and 6) that was responsible for the most severe stunting at 100, 200 and 300 L ha^{-1} when compared to the remaining solo adjuvant treatments. Destinaire™ solo with glufosinate-ammonium had the highest stunting percentage of all the treatments at 100 L ha^{-1} (Figure 6.12).

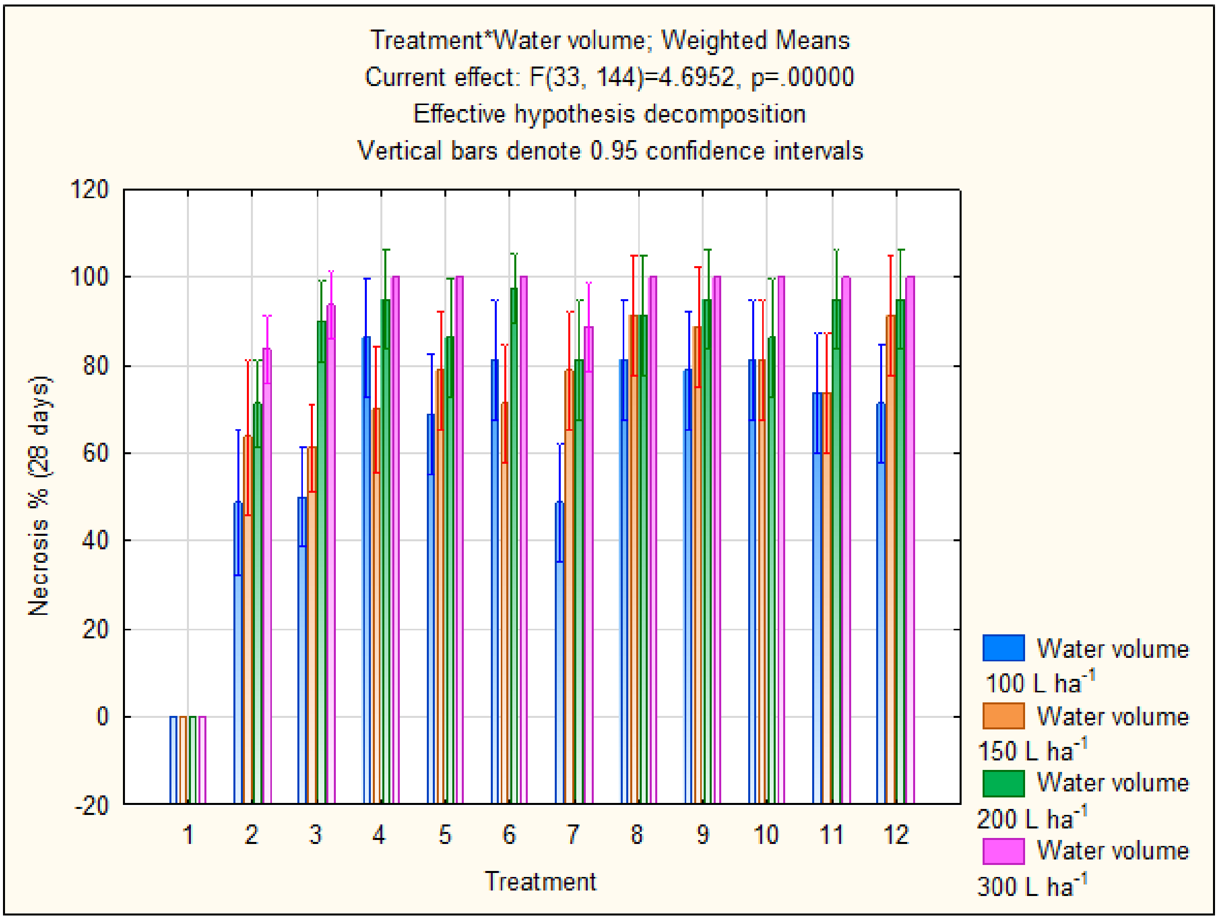


Figure 6.11: Effects of glufosinate-ammonium in combination with various adjuvants on necrosis of volunteer maize plants at 28 DAA, Nelspruit. (See Table 6.2 for treatment descriptions).

6.3.1.3 Mortality

A very clear trend arises when mortality is observed and a significant interaction between water volumes and treatments is present ($p<0.05$).

An increase in water volume led to a clear increase in mortality (Figure 6.13). The difference however between 200 and 300 L ha^{-1} is almost zero which was not expected. At 200 and 300 L ha^{-1} no clear differences arise between treatments, but at 100 and 150 L ha^{-1} there are noticeable differences.

Treatments 4, 5 and 6 resulted in the highest mortality rate for solo adjuvant mixtures at 100 L ha^{-1} (Figure 6.13). At 150 L ha^{-1} the same treatments stand out but Treatment 7, Interlock™ + glufosinate-ammonium also shows a high mortality rate at this water volume (Figure 6.13).

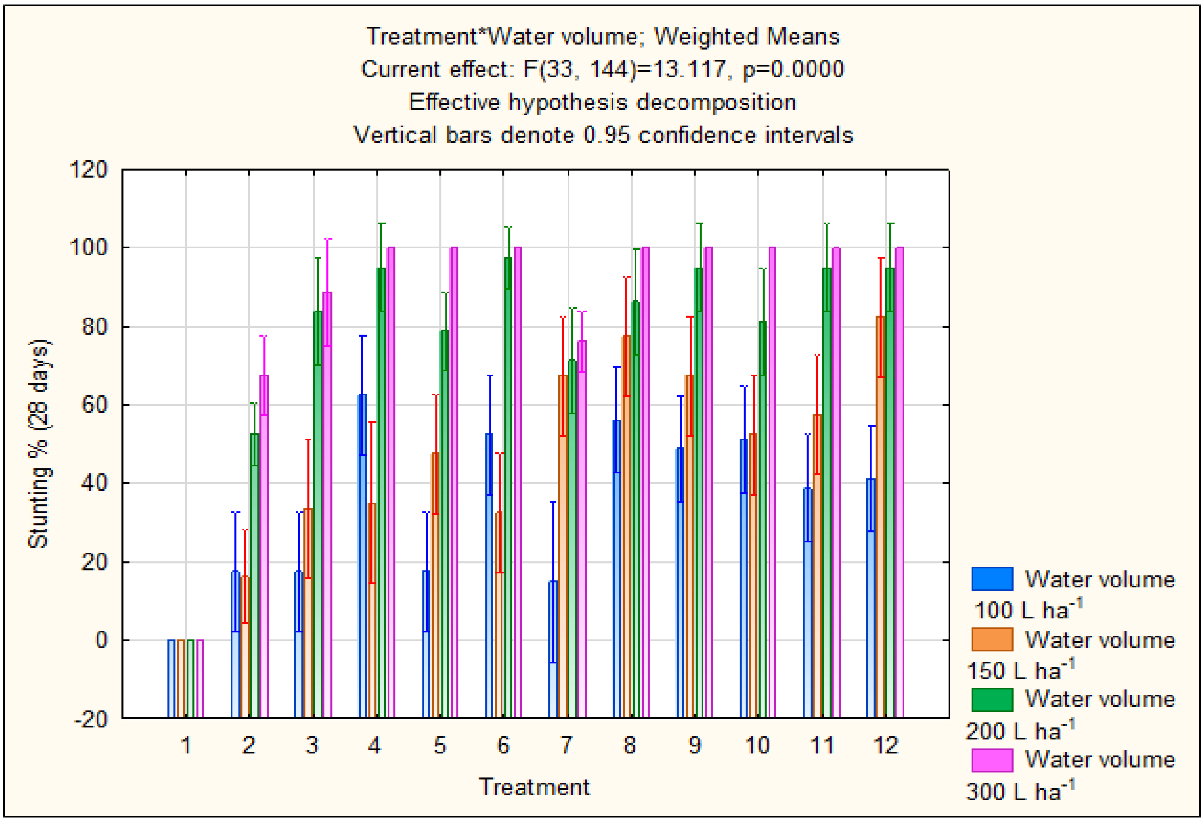


Figure 6.12: Effects of glufosinate-ammonium in combination with various adjuvants on stunting of volunteer maize plants at 28 DAA, Nelspruit. (See Table 6.2 for treatment descriptions).

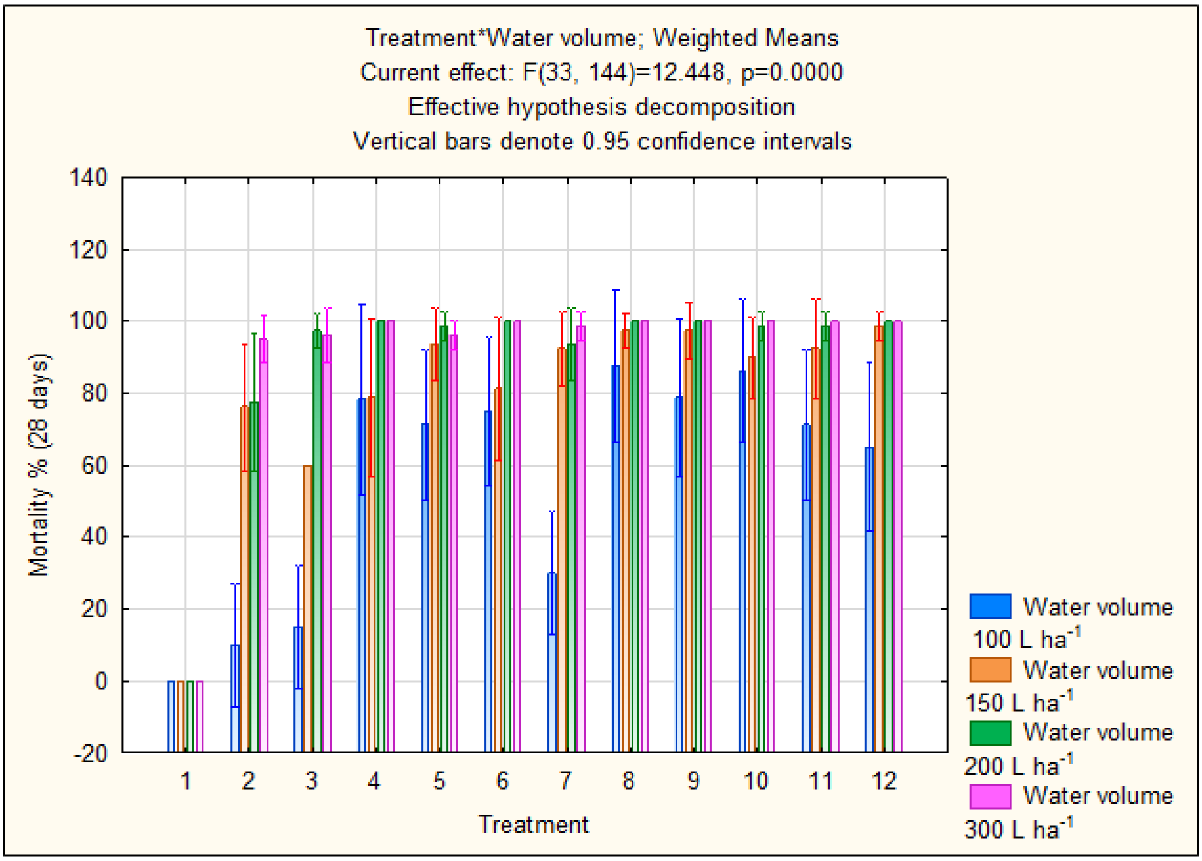


Figure 6.13*:* Effects of glufosinate-ammonium in combination with various adjuvants on percentage mortality of volunteer maize plants at 28 DAA, Nelspruit. (See Table 6.2 for treatment descriptions).

The 80% threshold analysis revealed exactly the same trends for necrosis, stunting and mortality. The figures are almost identical and therefore one figure will be provided that contains the 80% threshold for all three of those parameters (Figure 6.14).

The 80% threshold analysis revealed that 300 L ha^{-1} produced the shortest amount of time to reach the threshold for all three parameters. The adjuvant combination treatments, Treatments 8-12, all reached the 80% threshold in exactly 14 days at the 300 L ha^{-1} treatment (Figure 6.14). Class Act NG™ + glufosinate-ammonium, Treatment 6, was numerically the first solo adjuvant treatment to reach the 80% threshold at 200 and 300 L ha^{-1} (Figure 6.14).

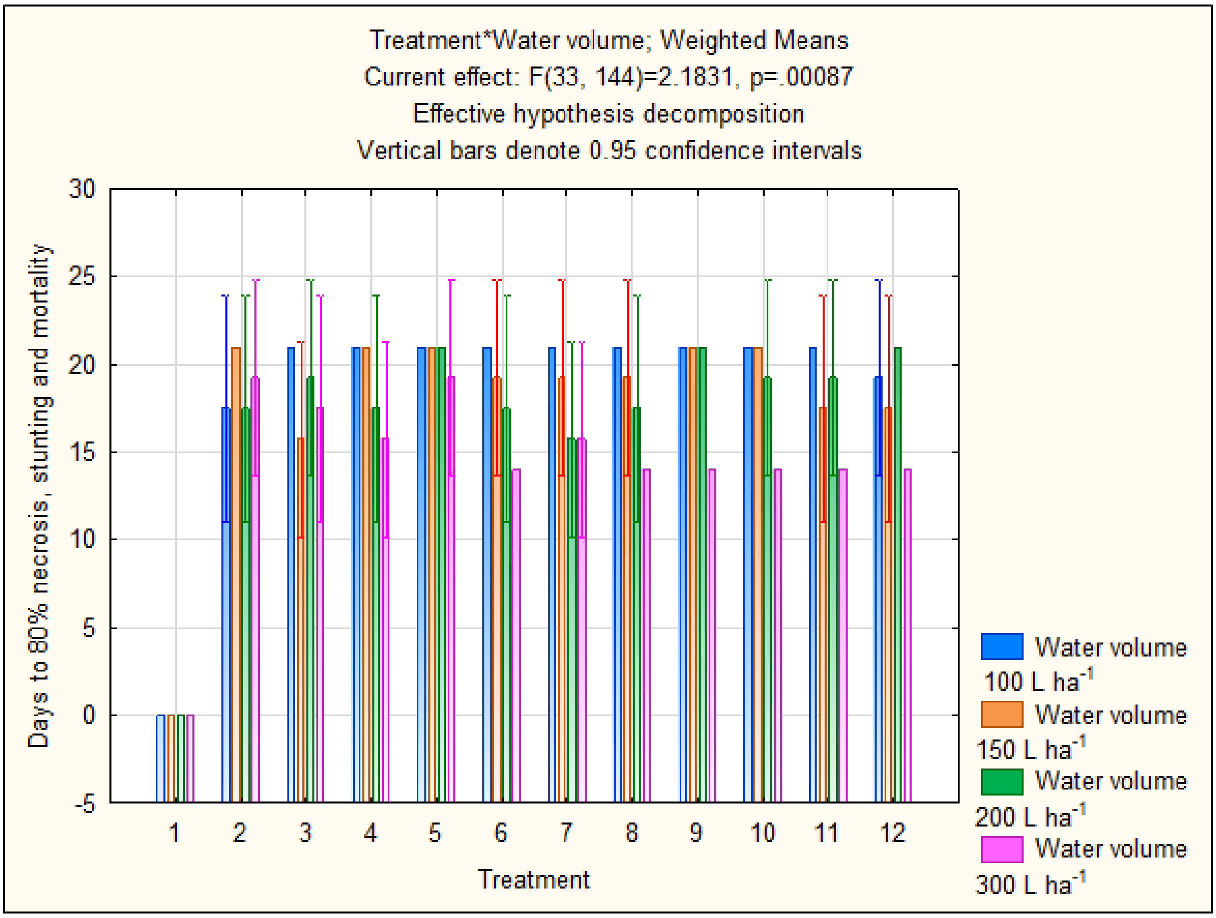


Figure 6.14: The effects of different adjuvants added to glufosinate-ammonium on days to 80% necrosis, stunting and mortality threshold of volunteer maize plants at 28 DAA, Nelspruit. (See Table 6.2 for treatment descriptions).

6.3.1.4 Coverage

A significant interaction was observed between treatments and water volumes ($p<0.05$). The expected increase in coverage with an increase in water volume did not occur as is evident in Figure 6.15. Treatment 6, Class Act NG™ + glufosinate-ammonium, did produce some consistency. At 300 L ha^{-1} treatments 6 provided the highest percentage coverage at this water volume with 36.7% (Figure 6.15). Treatment 6 caused 36.7% coverage at 100 L ha^{-1}

which is the second highest at this water volume and the highest solo adjuvant treatment at 100 L ha^{-1} (Figure 6.15).

The 150 L ha^{-1} application proved that Treatment 6 produced the highest percentage coverage of the solo adjuvant mixtures at 25% (Figure 6.15). Figure 6.15 revealed that 200 L ha^{-1} provided the lowest overall percentage coverage.

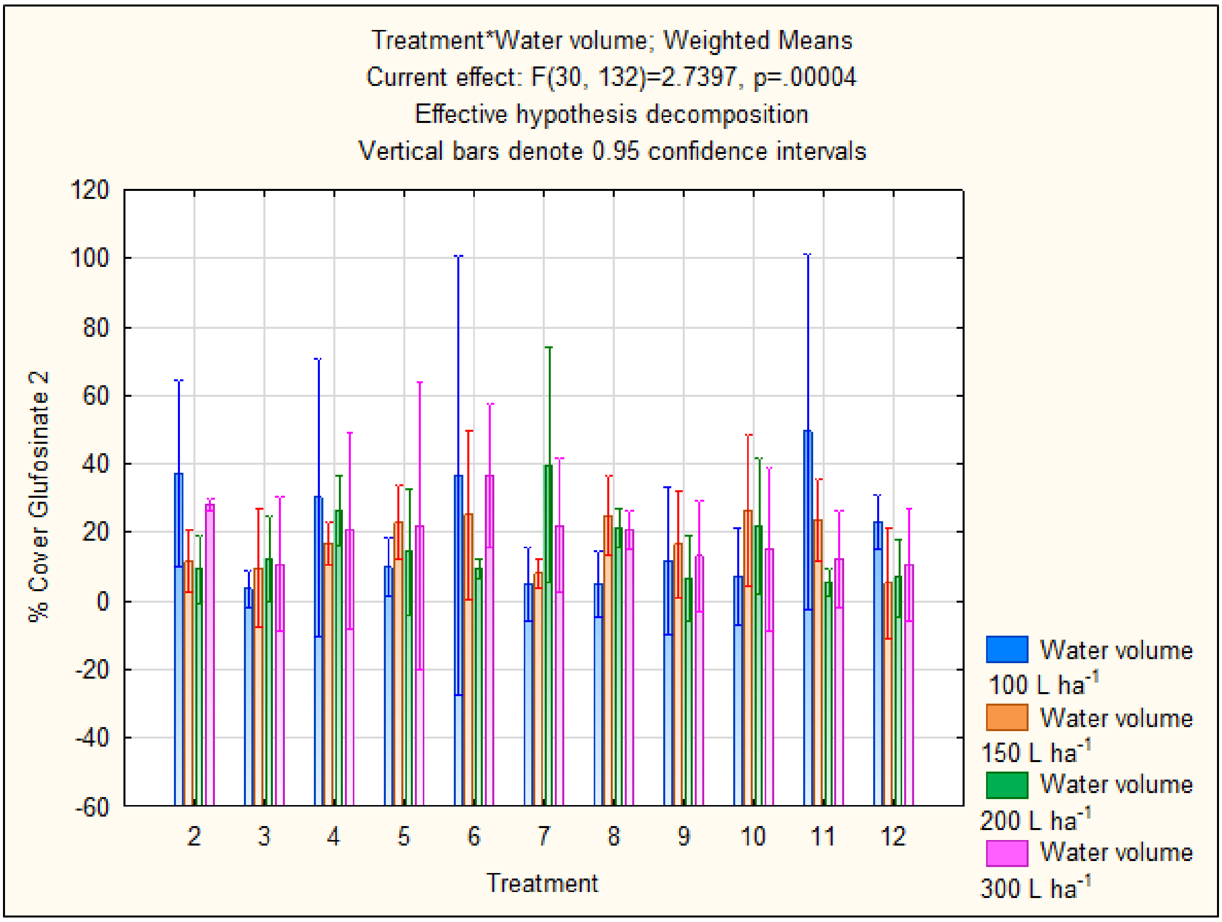


Figure 6.15: The effect of water volume on coverage of the herbicide-adjuvant mixtures on volunteer maize plants, Nelspruit. (See Table 6.2 for treatment descriptions).

6.3.1.3 Efficacy

The necrosis, stunting and mortality data at 28 DAA was used to determine the efficacy of herbicide-adjuvant mixtures to control glyphosate-resistant volunteer maize.

6.3.1.3.1 Necrosis

The analysis of the necrosis for efficacy revealed significant differences between treatments ($p<0.05$). Destinaire™ and Class Act NG™ were the adjuvants that provided great efficacy in causing necrosis in glyphosate-resistant volunteer maize.

Treatment 4 contained Destinaire™ + glufosinate-ammonium and caused 78,75% necrosis, similar to Treatment 6 (Figure 6.16). Treatment 6 had Class Act NG™ combined with glufosinate-ammonium and along with Treatment 4 these treatments produced not only the highest necrosis percentages for the solo adjuvant mixtures but also the second highest overall (Figure 6.16). Destinaire™ and Class Act NG™ was combined in Treatment 9, along with glufosinate-ammonium, to produce the highest overall necrosis with 82.5%. Treatments 4, 6 and 9 caused statistically higher necrosis percentages than the solo glufosinate-ammonium treatment, Treatment 2 (Figure 6.16).

6.3.1.3.2 Stunting

Class Act NG™ + glufosinate-ammonium (Treatment 6) caused the highest overall stunting percentage with 75% (Figure 6.17). This was numerically higher than any other treatment and statistically higher than Treatment 2, glufosinate-ammonium solo (Figure 6.17).

6.3.1.3.3 Mortality

Treatment 6, Class Act NG™ + glufosinate-ammonium, achieved 100% mortality at 28 DAA (Figure 6.18). This was numerically the highest mortality achieved but statistically only higher than Treatments 2 and 3, which contained glufosinate-ammonium solo and in combination with Direct (Treatment 3) (Figure 6.18).

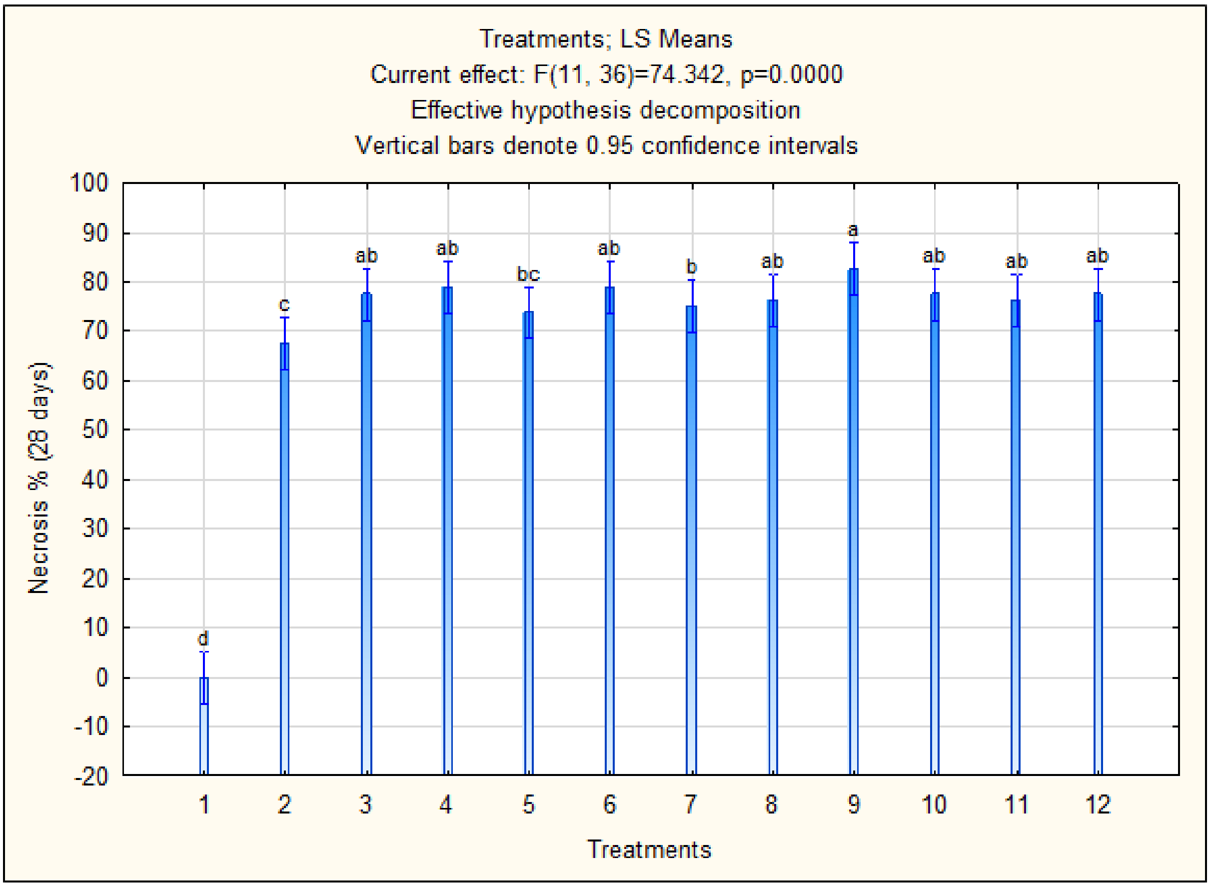


Figure 6.16: Effects of glufosinate-ammonium in combination with various adjuvants on necrosis of volunteer maize plants at 28 DAA, Nelspruit. (See Table 6.2 for treatment descriptions). Different letters at data points indicate significant differences between treatments at p = 0.05.

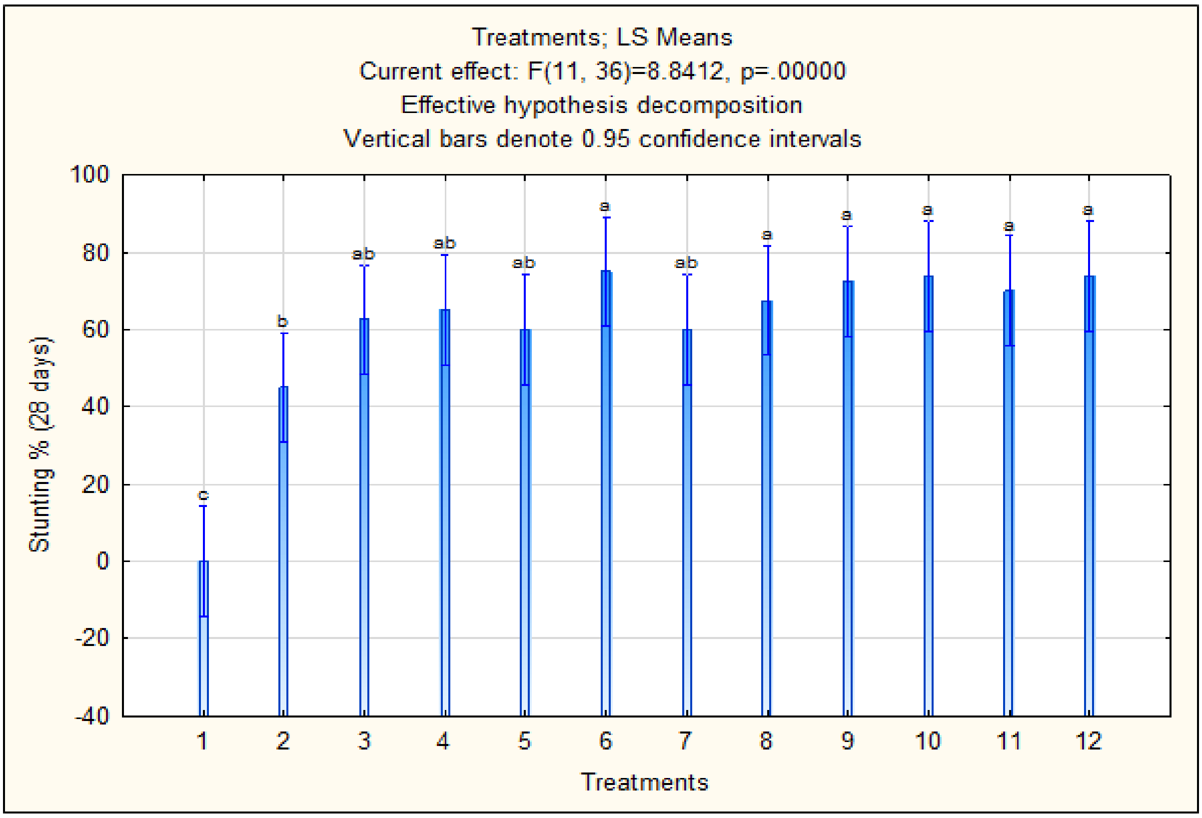


Figure 6.17: Effects of glufosinate-ammonium in combination with various adjuvants on stunting of volunteer maize plants at 28 DAA, Morgenzon. (See Table 6.2 for treatment descriptions). Different letters at data points indicate significant differences between treatments at p = 0.05.

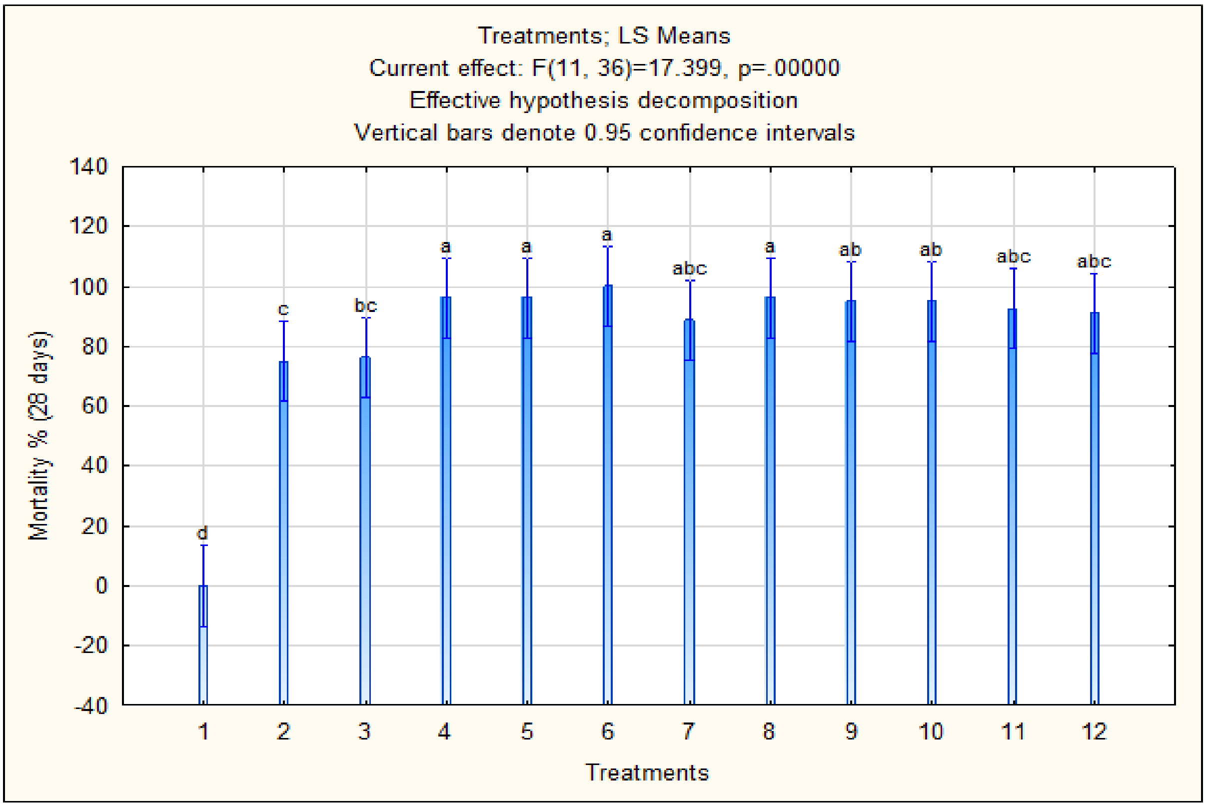


Figure 6.18: Effects of glufosinate-ammonium in combination with various adjuvants on mortality of volunteer maize plants at 28 DAA, Nelspruit. (See Table 6.2 for treatment descriptions). Different letters at data points indicate significant differences between treatments at p = 0.05.

6.4 Discussion

The trial sites delivered very interesting and similar results. Two matters are evident when the data from the two trial sites are observed. The first is the resounding influence of an increase in water volume on the increase of necrosis, stunting, mortality and coverage (Creech et al. 2015).

At 300 L ha^{-1} the highest necrosis percentages were observed at both Morgenzon (Figure 6.1) and Nelspruit (Figure 6.11). This trend continues when stunting is considered. The highest water volume, 300 L ha^{-1}, produced the most severe stunting of all the water volumes at both trial sites (Figures 6.3 and 6.12).

The mortality analysis revealed that there is a direct correlation between water volume and the efficacy of an herbicide-adjuvant mixture to control glyphosate-resistant volunteer maize. With an increase of water volume, the percentage mortality increased as well (Figures 6.5 and 6.13). This was expected and was proposed by Creech et al. (2015). The last evaluation of deposition, the coverage data, indicated that a higher water volume will lead to an increase in percentage cover (Figures 6.7 and 6.15) (Butts et al. 2018).

The second observation is the influence that Class Act NG™ had on the capability of glufosinate-ammonium to control glyphosate-resistant volunteer maize (Maschoff et al. 2000).

Class Act NG™ as a solo adjuvant mixture provided severe volunteer maize injury when necrosis and stunting is considered. As a solo adjuvant Class Act NG produced high necrosis percentages during the deposition and efficacy trials (Figures 6.8 and 6.16). The same can be said about stunting where Class Act NG™ provided high stunting percentages throughout the water volume range, including 100 L ha^{-1} (Figure 6.9). During the efficacy trials the same result was observed with Class Act NG™ being the solo adjuvant mixture that produced the highest stunting percentages (Figure 6.17). The mortality data supports the observations made during the necrosis and stunting evaluations where Class Act NG™ produced high mortality rates at both trial sites (Figures 6.10 and 6.18).

Class Act NG™ is successful in increasing the efficacy of glufosinate-ammonium due to the water conditioning role this adjuvant plays. Ammonium sulphate reduces ionic, specifically cation, interactions between the herbicide and those present in the carrier water (Travlos et al. 2017). This is important due to the fact that cations increase water hardness which decreases the efficacy of glufosinate-ammonium (Devkota and Johnson 2016).

The water hardness influences glufosinate-ammonium due to the characteristics of glufosinate-ammonium. Glufosinate-ammonium is a contact herbicide, which means all the interaction between the herbicide and the plant surface takes place on the outer part of the plant and translocation of the herbicide is not needed for the herbicide to cause weed injury (Carbonari et al. 2016). Because of the contact characteristic of glufosinate-ammonium it is of great importance to ensure the herbicide is not influenced by ionic interactions in the carrier water (Pline et al. 2000). Any ionic interaction between the herbicide and the carrier water will lead to a decrease in herbicide activity on the plant surface due to a decline in avalailable herbicide particles to interact with the plant surface (Jordan et al. 1989). Due to the nature of glufosinate-ammonium and the role ammonium sulphate plays in maximizing herbicide and plant interaction Class Act NG™ proved to be the adjuvant that most increases the efficacy of glufosinate-ammonium.

When the glufosinate-ammonium results are analysed it would seem that Class Act NG™ in combination with Destinaire™ (Treatment 9) and Interlock™ (Treatment 12) warrants special mention. The results these adjuvant combinations provided seem to indicate that for glufosinate-ammonium a combination of adjuvants will be beneficial. There is however no substantial evidence that an additional adjuvant will necessarily be economically viable because the differences, if there are any, between Class Act NG™ solo and in combination with Destinaire™ and Interlock™ were very rarely statistically significant.

From the above discussion the following conclusions can be made. The first is that it will be beneficial to apply glufosinate-ammonium, with any of the adjuvants, at 300 L ha^{-1} (Creech et al. 2015). The second is that Class Act NG™ is the adjuvant that had the most profound impact on the improved capability of glufosinate-ammonium to control glyphosate-resistant volunteer maize (Maschoff et al. 2000).

6.5 References

Butts TR, Samples CA, Franca LX, Dodds DM, Reynolds DB, Adams JW, Zollinger RK, Howatt KA, Fritz BK, Hoffmann WC, Kruger GR. 2018. Spray droplet size and carrier volume effect on dicamba and glufosinate efficacy. *Pest Management Science* 74: 2020-2029.

Carbonari CA, Latorre DO, Gomes GLGC, Velini ED, Owens DK, Pan Z, Dayan FE. 2016. Resistance to glufosinate is proportional to phosphinothricin acetyltransferase expression and activity in LibertyLink and WideStrike cotton. Planta 243: 925-933.

Creech CF, Henry RS, Werle R, Sandell LD. 2015. Performance of postemergence herbicides applied at different carrier volume rates. *Weed Technology* 29: 611-624.

Devkota P, Johnson WG. 2016. Glufosinate efficacy as influenced by carrier water pH, hardness, foliar fertilizer and ammonium sulfate. *Weed Technology* 30: 848-859.

Jordan DL, York AC, Corbin FT. 1989. Effect of ammonium sulfate and bentazon on sethoxydim absorption. Weed Technology 3: 674-677

Maschhoff JR, Hart SE, Baldwin JL. 2000. Effect of ammonium sulfate on the efficacy, absorption, and translocation of glufosinate. *Weed Technology* 48: 2-6.

Pline WA, Hatzios KK, Hagood ES. 2000. Weed and herbicide-resistant soybean (*Glycine max*) response to glufosinate and glyphosate plus ammonium sulfate and pelargonic acid. *Weed Technology* 14: 667-674.

Travlos I, Cheimona N, Bilalis D. 2017. Glyphosate efficacy of different salt formulations and adjuvant additives on various weeds. Agronomy 7: 1-9.

Chapter seven- General conclusion

The aim of this thesis was to determine the efficacy of selected herbicide-adjuvant mixtures for the control of Roundup Ready (glyphosate-resistant) volunteer maize, and in my opinion that main objective was reached.

This paper determined that the optimal application water volume for maximum efficacy is 300 L ha^{-1} . It was further determined that Destinaire™ and Summit Super are the adjuvants that compliment quizalofop-P-tefuryl the most.

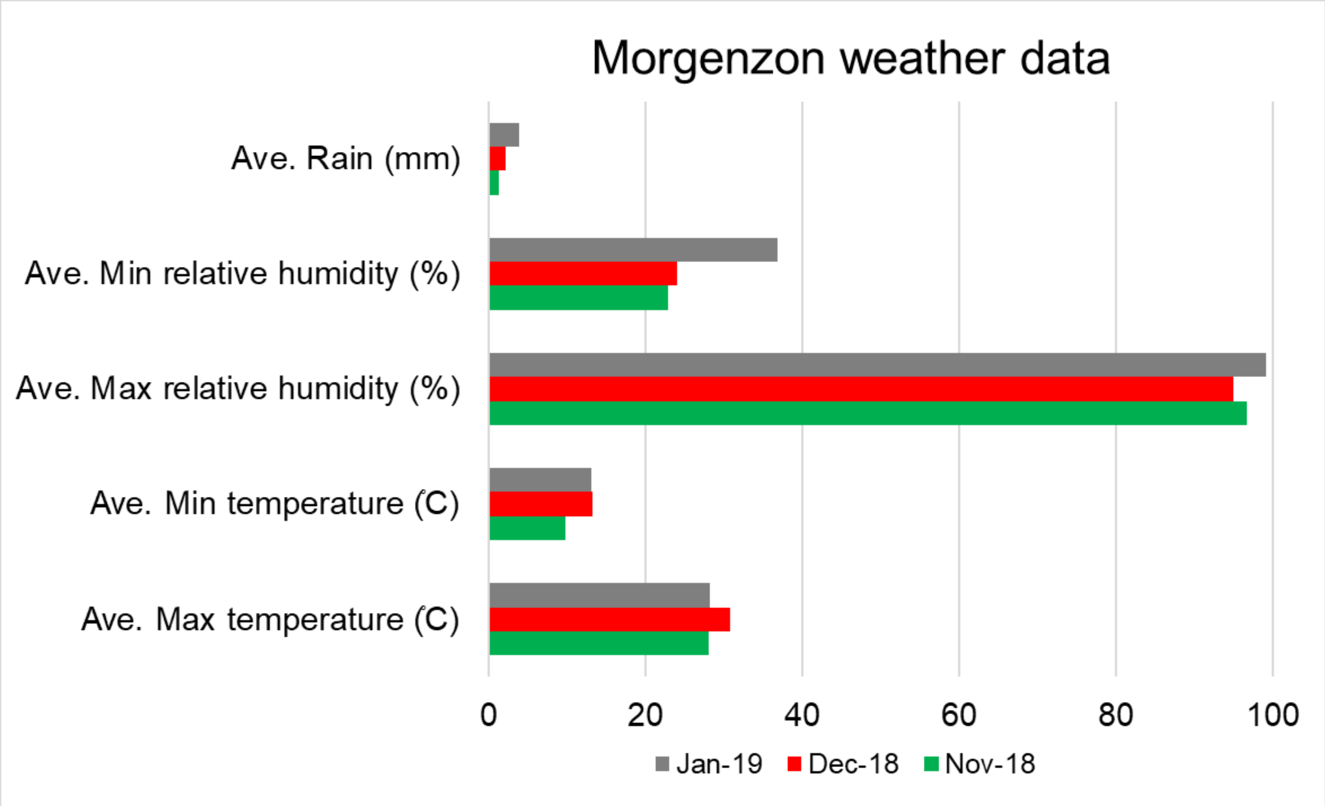
Destinaire™ was also the adjuvant of choice to improve the efficacy of clethodim in controlling glyphosate-resistant volunteer maize. Class Act NG™ proved to aid glufosinate-ammonium the most in controlling glyphosate-resistant volunteer maize.

The coverage data did not yield the expected outcomes and it was rather disappointing because great expectation was placed on the coverage data to produce insights into the effect of adjuvants and to why they aid herbicides. Unfortunately, the reason for the underwhelming data is unknown and because of this it would be my recommendation that future studies repeat the coverage experiments but in a more controlled environment.

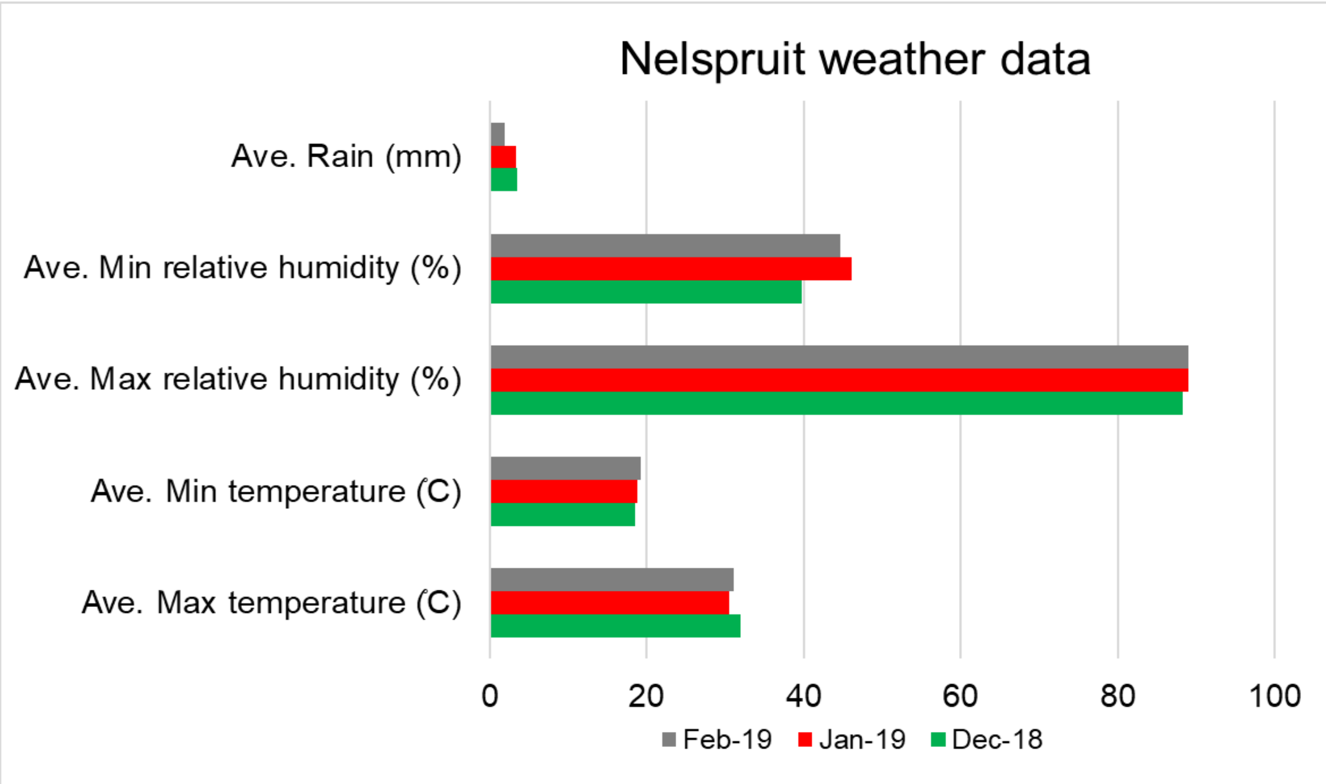
If the coverage experiment were to be repeated, I would recommend it be done in a controlled environment where the wind especially does not have an influence. A large barn or warehouse will be ideal as no crop or plant is needed.

The last conclusion and advise to future studies I can make is to determine the financial impacts that an increase in water volume and the addition of adjuvants will have on the farmer. Farming, in the end, is still a business and unaffordable solutions will not aid the farmer nor will the problem be solved.

Annexure A:



Weather data as provided by the ARC for the Morgenzon trial site.



Weather data as provided by the ARC for the Nelspruit trial site.

Annexure 1. Anova tables from Chapter 4:

Table 1.1 Anova table for deposition necrosis, Morgenzon.

Univariate Tests of Significance for Necrosis % 28 (Quizalofop 1 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 8.5284

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	248244,9	1	248244,9	3413,068	0,00
Treatment	81517,0	13	6270,5	86,212	0,00
Water volume	71609,0	3	23869,7	328,179	0,00
Treatment*Water volume	70938,9	39	1818,9	25,008	0,00
Error	12219,2	168	72,7		

Table 1.2 Anova table for 80% necrosis, Morgenzon.

Univariate Tests of Significance for Necrosis Days to 80% (Quizalofop 1 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 5.5340

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	56896,88	1	56896,88	1857,857	0,000000
Treatment	5388,25	13	414,48	13,534	0,000000
Water volume	2270,63	3	756,88	24,714	0,000000
Treatment*Water volume	2329,25	39	59,72	1,950	0,001995
Error	5145,00	168	30,63		

Table 1.3 Anova table for deposition stunting, Morgenzon.

Univariate Tests of Significance for Stunting % 28 (Quizalofop 1 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 5.2342

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	37208,79	1	37208,79	1358,118	0,00
Treatment	15376,90	13	1182,84	43,173	0,00
Water volume	13300,48	3	4433,49	161,822	0,00
Treatment*Water volume	18010,08	39	461,80	16,856	0,00
Error	4602,75	168	27,40		

Table 1.4 Anova table for 80% stunting, Morgenzon.

Univariate Tests of Significance for Stunting Days to 80% (Quizalofop 1 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 6.2283

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	43512,87	1	43512,87	1121,707	0,000000
Treatment	5628,00	13	432,92	11,160	0,000000
Water volume	5376,87	3	1792,29	46,203	0,000000
Treatment*Water volume	2665,25	39	68,34	1,762	0,007614
Error	6517,00	168	38,79		

Table 1.5 Anova table for deposition mortality, Morgenzon.

Univariate Tests of Significance for Mortality % 28 (Quizalofop 1 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 14.2705

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	569036,2	1	569036,2	2794,244	0,000000
Treatment	97770,1	13	7520,8	36,931	0,000000
Water volume	70945,1	3	23648,4	116,125	0,000000
Treatment*Water volume	43036,2	39	1103,5	5,419	0,000000
Error	34212,5	168	203,6		

Table 1.6 Anova table for 80% mortality, Morgenzon.

Univariate Tests of Significance for Mortality days to 80% (Quizalofop 1 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 5.1092

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	58243,50	1	58243,50	2231,196	0,000000
Treatment	4801,13	13	369,32	14,148	0,000000
Water volume	1874,25	3	624,75	23,933	0,000000
Treatment*Water volume	1549,62	39	39,73	1,522	0,036729
Error	4385,50	168	26,10		

Table 1.7 Anova table for coverage, Morgenzon.

Univariate Tests of Significance for % Cover Quizalofop1 (QUIZAFOLOP in DATA Coverage 20190724)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 11.7808

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	92626,85	1	92626,85	667,3964	0,000000
Treatment	8386,00	12	698,83	5,0352	0,000000
Water volume	13036,61	3	4345,54	31,3105	0,000000
Treatment*Water volume	16312,12	36	453,11	3,2648	0,000000
Error	21650,98	156	138,79		

Table 1.8 Anova table for efficacy necrosis, Morgenzon.

Levene's Test for Homogeneity of Variances (Quizalofop 1 28 DAA in DATA Mortality 28 DAA data 20190806)
Effect: "Treatments"
Degrees of freedom for all F's: 13, 42

	MS Effect	MS Error	F	p
Necrosis % 28	619,8269	27,59821	22,45895	0,000000

Table 1.9 Anova table for efficacy stunting, Morgenzon.

Levene's Test for Homogeneity of Variances (Quizalofop 1 28 DAA in DATA Mortality 28 DAA data 20190806)
Effect: "Treatments"
Degrees of freedom for all F's: 13, 42

	MS Effect	MS Error	F	p
Stunting % 28	38,94677	6,135417	6,347861	0,000002

Table 1.10 Anova table for efficacy mortality, Morgenzon.

Levene's Test for Homogeneity of Variances (Quizalofop 1 28 DAA in DATA Mortality 28 DAA data 20190806)
Effect: "Treatments"
Degrees of freedom for all F's: 13, 42

	MS Effect	MS Error	F	p
Mortality % 28	408,5937	117,2991	3,483349	0,001048

Table 1.11 Anova table for deposition necrosis, Nelspruit.

Univariate Tests of Significance for Necrosis % 28 (Quizalofop 2 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 9.4589

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	606424,2	1	606424,2	6777,831	0,00
Treatment	100111,7	13	7700,9	86,071	0,00
Water volume	33976,2	3	11325,4	126,581	0,00
Treatment*Water volume	24381,6	39	625,2	6,987	0,00
Error	15031,3	168	89,5		

Table 1.12 Anova table for 80% necrosis, Nelspruit.

Univariate Tests of Significance for Necrosis Days to 80% (Quizalofop 2 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 3.1258

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	68600,00	1	68600,00	7020,896	0,000000
Treatment	5445,13	13	418,86	42,868	0,000000
Water volume	194,25	3	64,75	6,627	0,000294
Treatment*Water volume	755,12	39	19,36	1,982	0,001585
Error	1641,50	168	9,77		

Table 1.13 Anova table for deposition stunting, Nelspruit.

Univariate Tests of Significance for Stunting % 28 (Quizalofop 2 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 6.0746

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	66275,04	1	66275,04	1796,057	0,00
Treatment	27340,52	13	2103,12	56,995	0,00
Water volume	27538,98	3	9179,66	248,769	0,00
Treatment*Water volume	22827,21	39	585,31	15,862	0,00
Error	6199,25	168	36,90		

Table 1.14 Anova table for 80% stunting, Nelspruit.

Univariate Tests of Significance for Stunting Days to 80% (Quizalofop 2 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 5.9682

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	47736,16	1	47736,16	1340,186	0,000000
Treatment	4787,96	13	368,30	10,340	0,000000
Water volume	4691,23	3	1563,74	43,902	0,000000
Treatment*Water volume	3294,64	39	84,48	2,372	0,000082
Error	5984,00	168	35,62		

Table 1.15 Anova table for deposition mortality, Nelspruit.

Univariate Tests of Significance for Mortality % 28 (Quizalofop 2 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 10.8030

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	646612,6	1	646612,6	5540,627	0,00
Treatment	180657,7	13	13896,7	119,077	0,00
Water volume	79570,0	3	26523,3	227,270	0,00
Treatment*Water volume	49828,5	39	1277,7	10,948	0,00
Error	19606,2	168	116,7		

Table 1.16 Anova table for 80% mortality, Nelspruit.

Univariate Tests of Significance for Mortality days to 80% (Quizalofop 2 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 5.7579

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	54531,36	1	54531,36	1644,826	0,000000
Treatment	4552,83	13	350,22	10,564	0,000000
Water volume	299,58	3	99,86	3,012	0,031671
Treatment*Water volume	1837,48	39	47,11	1,421	0,067280
Error	5569,75	168	33,15		

Table 1.17 Anova table for coverage, Nelspruit.

Univariate Tests of Significance for % Cover Quizalofop2 (QUIZAFOLOP in DATA Coverage 20190724)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 15.5262

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	121438,0	1	121438,0	503,7583	0,000000
Treatment	9275,6	12	773,0	3,2065	0,000395
Water volume	10700,8	3	3566,9	14,7966	0,000000
Treatment*Water volume	17577,6	36	488,3	2,0255	0,001664
Error	37606,0	156	241,1		

Table 1.18 Anova table for efficacy necrosis, Nelspruit.

	Levene's Test for Homogeneity of Variances (Quizalofop 2 28 DAA in DATA Mortality 28 DAA data 20190806) Effect: "Treatments" Degrees of freedom for all F's: 13, 42			
	MS Effect	MS Error	F	p
Necrosis % 28	101,8832	24,81845	4,105141	0,000238

Table 1.19 Anova table for efficacy stunting, Nelspruit.

	Levene's Test for Homogeneity of Variances (Quizalofop 2 28 DAA in DATA Mortality 28 DAA data 20190806) Effect: "Treatments" Degrees of freedom for all F's: 13, 42			
	MS Effect	MS Error	F	p
Stunting % 28	6,472871	1,638393	3,950744	0,000341

Table 1.20 Anova table for efficacy mortality, Nelspruit.

	Levene's Test for Homogeneity of Variances (Quizalofop 2 28 DAA in DATA Mortality 28 DAA data 20190806) Effect: "Treatments" Degrees of freedom for all F's: 13, 42			
	MS Effect	MS Error	F	p
Mortality % 28	62,70604	22,24702	2,818626	0,005514

Annexure 2. Anova tables from Chapter 5:

Table 2.1 Anova table for deposition necrosis, Morgenzon.

Univariate Tests of Significance for Necrosis % 28 (Clethodim 1 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 9.2700

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	871276,2	1	871276,2	10139,05	0,00
Treatment	147822,2	12	12318,5	143,35	0,00
Water volume	14038,7	3	4679,6	54,46	0,00
Treatment*Water volume	23811,4	36	661,4	7,70	0,00
Error	13405,5	156	85,9		

Table 2.2 Anova table for 80% necrosis, Morgenzon.

Univariate Tests of Significance for Necrosis Days to 80% (Clethodim 1 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 3.2195

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	59366,33	1	59366,33	5727,364	0,000000
Treatment	5227,92	12	435,66	42,030	0,000000
Water volume	117,79	3	39,26	3,788	0,011706
Treatment*Water volume	800,96	36	22,25	2,146	0,000715
Error	1617,00	156	10,37		

Table 2.3 Anova table for deposition stunting, Morgenzon.

Univariate Tests of Significance for Stunting % 28 (Clethodim 1 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 9.8218

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	261280,7	1	261280,7	2708,472	0,000000
Treatment	43035,6	12	3586,3	37,176	0,000000
Water volume	8224,7	3	2741,6	28,420	0,000000
Treatment*Water volume	11034,0	36	306,5	3,177	0,000000
Error	15049,0	156	96,5		

Table 2.4 Anova table for 80% stunting, Morgenzon.

Univariate Tests of Significance for Stunting Days to 80% (Clethodim 1 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 3.4320

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	69204,02	1	69204,02	5875,280	0,000000
Treatment	6249,86	12	520,82	44,217	0,000000
Water volume	8,48	3	2,83	0,240	0,868339
Treatment*Water volume	512,14	36	14,23	1,208	0,215288
Error	1837,50	156	11,78		

Table 2.5 Anova table for deposition mortality, Morgenzon.

Univariate Tests of Significance for Mortality % 28 (Clethodim 1 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 9.4754

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	1279708	1	1279708	14253,24	0,000000
Treatment	181162	12	15097	168,15	0,000000
Water volume	6284	3	2095	23,33	0,000000
Treatment*Water volume	17964	36	499	5,56	0,000000
Error	14006	156	90		

Table 2.6 Anova table for 80% mortality, Morgenzon.

Univariate Tests of Significance for Mortality days to 80% (Clethodim 1 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 3.5446

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	49848,08	1	49848,08	3967,500	0,000000
Treatment	4511,30	12	375,94	29,922	0,000000
Water volume	33,92	3	11,31	0,900	0,442706
Treatment*Water volume	486,70	36	13,52	1,076	0,368411
Error	1960,00	156	12,56		

Table 2.7 Anova table for coverage, Morgenzon.

Univariate Tests of Significance for % Cover Clethodim 1 (CLEHTODIM in DATA Coverage 20190724)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 13.8520

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	106798,3	1	106798,3	556,5956	0,000000
Treatment	4657,0	11	423,4	2,2064	0,017019
Water volume	16008,5	3	5336,2	27,8102	0,000000
Treatment*Water volume	22104,0	33	669,8	3,4909	0,000000
Error	27630,4	144	191,9		

Table 2.8 Anova table for efficacy necrosis, Morgenzon.

Univariate Tests of Significance for Necrosis % 28 (Clethodim 1 28 DAA in DATA Mortality 28 DAA data 20190806)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 21.0774

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	153364,9	1	153364,9	345,2171	0,000000
Treatments	49897,1	12	4158,1	9,3597	0,000000
Error	17326,0	39	444,3		

Table 2.9 Anova table for efficacy stunting, Morgenzon.

Univariate Tests of Significance for Stunting % 28 (Clethodim 1 28 DAA in DATA Mortality 28 DAA data 20190806)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 21.6697

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	85293,00	1	85293,00	181,6380	0,000000
Treatments	45631,50	12	3802,63	8,0980	0,000000
Error	18313,50	39	469,58		

Table 2.10 Anova table for efficacy mortality, Morgenzon.

Univariate Tests of Significance for Mortality % 28 (Clethodim 1 28 DAA in DATA Mortality 28 DAA data 20190806)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 14.8443

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	251312,0	1	251312,0	1140,500	0,000000
Treatments	49869,2	12	4155,8	18,860	0,000000
Error	8593,7	39	220,4		

Table 2.11 Anova table for deposition necrosis, Nelspruit.

Univariate Tests of Significance for Necrosis % 28 (Clethodim 2 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 7.9362

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	1012491	1	1012491	16075,38	0,00
Treatment	118612	12	9884	156,93	0,00
Water volume	37058	3	12353	196,12	0,00
Treatment*Water volume	17267	36	480	7,62	0,00
Error	9826	156	63		

Table 2.12 Anova table for 80% necrosis, Nelspruit.

Univariate Tests of Significance for Necrosis Days to 80% (Clethodim 2 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 2.9523

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	60554,81	1	60554,81	6947,270	0,000000
Treatment	5316,50	12	443,04	50,829	0,000000
Water volume	232,51	3	77,50	8,892	0,000018
Treatment*Water volume	597,42	36	16,60	1,904	0,003809
Error	1359,75	156	8,72		

Table 2.13 Anova table for deposition stunting, Nelspruit.

Univariate Tests of Significance for Stunting % 28 (Clethodim 2 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 6.0828

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	422460,9	1	422460,9	11417,86	0,00
Treatment	106548,2	12	8879,0	239,97	0,00
Water volume	172752,6	3	57584,2	1556,33	0,00
Treatment*Water volume	84084,3	36	2335,7	63,13	0,00
Error	5772,0	156	37,0		

Table 2.14 Anova table for 80% stunting, Nelspruit.

Univariate Tests of Significance for Stunting Days to 80% (Clethodim 2 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 4.8698

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	57489,25	1	57489,25	2424,199	0,000000
Treatment	5892,25	12	491,02	20,705	0,000000
Water volume	1587,79	3	529,26	22,318	0,000000
Treatment*Water volume	2773,21	36	77,03	3,248	0,000000
Error	3699,50	156	23,71		

Table 2.15 Anova table for deposition mortality, Nelspruit.

Univariate Tests of Significance for Mortality % 28 (Clethodim 2 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 7.2141

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	1092575	1	1092575	20993,59	0,00
Treatment	175633	12	14636	281,23	0,00
Water volume	40674	3	13558	260,52	0,00
Treatment*Water volume	44024	36	1223	23,50	0,00
Error	8119	156	52		

Table 2.16 Anova table for 80% mortality, Nelspruit.

Univariate Tests of Significance for Mortality days to 80% (Clethodim 2 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 4.2866

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	53825,56	1	53825,56	2929,282	0,000000
Treatment	4888,69	12	407,39	22,171	0,000000
Water volume	57,48	3	19,16	1,043	0,375400
Treatment*Water volume	1081,77	36	30,05	1,635	0,021516
Error	2866,50	156	18,37		

Table 2.17 Anova table for coverage, Nelspruit.

Univariate Tests of Significance for % Cover Clethodim 2 (CLEHTODIM in DATA Coverage 20190724)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 16.0443

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	99178,04	1	99178,04	385,2790	0,000000
Treatment	4888,77	11	444,43	1,7265	0,073023
Water volume	12911,62	3	4303,87	16,7193	0,000000
Treatment*Water volume	26912,47	33	815,53	3,1681	0,000001
Error	37068,30	144	257,42		

Table 2.18 Anova table for efficacy necrosis, Nelspruit.

Univariate Tests of Significance for Necrosis % 28 (Clethodim 2 28 DAA in DATA Mortality 28 DAA data 20190806)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 11.5822

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	192882,5	1	192882,5	1437,839	0,000000
Treatments	29514,8	12	2459,6	18,335	0,000000
Error	5231,7	39	134,1		

Table 2.19 Anova table for efficacy stunting, Nelspruit.

Univariate Tests of Significance for Stunting % 28 (Clethodim 2 28 DAA in DATA Mortality 28 DAA data 20190806)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 24.5170

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	23651,56	1	23651,56	39,34822	0,000000
Treatments	17959,19	12	1496,60	2,48984	0,015718
Error	23442,25	39	601,08		

Table 2.20 Anova table for efficacy mortality, Nelspruit.

Univariate Tests of Significance for Mortality % 28 (Clethodim 2 28 DAA in DATA Mortality 28 DAA data 20190806)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 15.1964

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	237600,5	1	237600,5	1028,888	0,000000
Treatments	48918,3	12	4076,5	17,653	0,000000
Error	9006,3	39	230,9		

Annexure 3. Anova tables for Chapter 6:

Table 3.1 Anova table for deposition necrosis, Morgenzon.

Univariate Tests of Significance for Necrosis % 28 (Glufosinate 1 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 6.6536

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	735075,0	1	735075,0	16604,05	0,00
Treatment	93903,1	11	8536,6	192,83	0,00
Water volume	116476,0	3	38825,3	877,00	0,00
Treatment*Water volume	32920,8	33	997,6	22,53	0,00
Error	6375,0	144	44,3		

Table 3.2 Anova table for 80% necrosis, Morgenzon.

Univariate Tests of Significance for Necrosis Days to 80% (Glufosinate 1 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 3.2998

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	48070,02	1	48070,02	4414,594	0,000000
Treatment	4445,73	11	404,16	37,116	0,000000
Water volume	311,35	3	103,78	9,531	0,000009
Treatment*Water volume	190,90	33	5,78	0,531	0,982136
Error	1568,00	144	10,89		

Table 3.3 Anova table for deposition stunting, Morgenzon.

Univariate Tests of Significance for Stunting % 28 (Glufosinate 1 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 7.1291

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	211006,4	1	211006,4	4151,654	0,000000
Treatment	29076,4	11	2643,3	52,008	0,000000
Water volume	40851,4	3	13617,1	267,924	0,000000
Treatment*Water volume	10422,0	33	315,8	6,214	0,000000
Error	7318,8	144	50,8		

Table 3.4 Anova table for 80% stunting, Morgenzon.

Univariate Tests of Significance for Stunting Days to 80% (Glufosinate 1 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 4.5185

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	49408,33	1	49408,33	2420,000	0,000000
Treatment	4669,29	11	424,48	20,791	0,000000
Water volume	320,54	3	106,85	5,233	0,001857
Treatment*Water volume	1069,83	33	32,42	1,588	0,033974
Error	2940,00	144	20,42		

Table 3.5 Anova table for deposition mortality, Morgenzon.

Univariate Tests of Significance for Mortality % 28 (Glufosinate 1 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 8.1729

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	824907,4	1	824907,4	12349,49	0,00
Treatment	97519,1	11	8865,4	132,72	0,00
Water volume	127934,8	3	42644,9	638,43	0,00
Treatment*Water volume	26194,9	33	793,8	11,88	0,00
Error	9618,7	144	66,8		

Table 3.6 Anova table for 80% mortality, Morgenzon.

Univariate Tests of Significance for Mortality days to 80% (Glufosinate 1 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 4.4616

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	48514,08	1	48514,08	2437,128	0,000000
Treatment	4693,79	11	426,71	21,436	0,000000
Water volume	151,08	3	50,36	2,530	0,059597
Treatment*Water volume	1202,54	33	36,44	1,831	0,008191
Error	2866,50	144	19,91		

Table 3.7 Anova table for coverage, Morgenzon.

Univariate Tests of Significance for % Cover Glufosinate 1 (GLUFOSINATE in DATA Coverage 20190724)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 11.2045

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	69344,63	1	69344,63	552,3682	0,000000
Treatment	3977,97	10	397,80	3,1687	0,001140
Water volume	5087,70	3	1695,90	13,5088	0,000000
Treatment*Water volume	10976,69	30	365,89	2,9145	0,000015
Error	16571,36	132	125,54		

Table 3.8 Anova table for efficacy necrosis, Morgenzon.

Univariate Tests of Significance for Necrosis % 28 (Glufosinate 1 28 DAA in DATA Mortality 28 DAA data 20190806)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 18.0181

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	280602,1	1	280602,1	864,3144	0,000000
Treatments	30010,4	11	2728,2	8,4035	0,000000
Error	11687,5	36	324,7		

Table 3.9 Anova table for efficacy stunting, Morgenzon.

Univariate Tests of Significance for Stunting % 28 (Glufosinate 1 28 DAA in DATA Mortality 28 DAA data 20190806)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 22.3734

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	211736,3	1	211736,3	422,9909	0,000000
Treatments	26287,2	11	2389,7	4,7740	0,000168
Error	18020,5	36	500,6		

Table 3.10 Anova table for efficacy mortality, Morgenzon.

Univariate Tests of Significance for Mortality % 28 (Glufosinate 1 28 DAA in DATA Mortality 28 DAA data 20190806)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 19.2706

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	317688,0	1	317688,0	855,4853	0,000000
Treatments	32118,2	11	2919,8	7,8627	0,000001
Error	13368,7	36	371,4		

Table 3.11 Anova table for deposition necrosis, Nelspruit.

Univariate Tests of Significance for Necrosis % 28 (Glufosinate 2 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 6.9472

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	1122408	1	1122408	23255,65	0,000000
Treatment	112404	11	10219	211,72	0,000000
Water volume	17609	3	5870	121,62	0,000000
Treatment*Water volume	7478	33	227	4,70	0,000000
Error	6950	144	48		

Table 3.12 Anova table for 80% necrosis, Nelspruit.

Univariate Tests of Significance for Necrosis Days to 80% (Glufosinate 2 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 2.4576

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	55658,13	1	55658,13	9215,028	0,000000
Treatment	5166,18	11	469,65	77,758	0,000000
Water volume	541,81	3	180,60	29,901	0,000000
Treatment*Water volume	435,13	33	13,19	2,183	0,000872
Error	869,75	144	6,04		

Table 3.13 Anova table for deposition stunting, Nelspruit.

Univariate Tests of Significance for Stunting % 28 (Glufosinate 2 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 7.6206

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	726438,(	1	726438,(	12509,07	0,00
Treatment	91871,4	11	8351,9	143,82	0,00
Water volume	84740,1	3	28246,7	486,40	0,00
Treatment*Water volume	25138,(	33	761,8	13,12	0,00
Error	8362,5	144	58,1		

Table 3.14 Anova table for 80% stunting, Nelspruit.

Univariate Tests of Significance for Stunting Days to 80% (Glufosinate 2 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 2.4576

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	55658,13	1	55658,13	9215,028	0,000000
Treatment	5166,18	11	469,65	77,758	0,000000
Water volume	541,81	3	180,60	29,901	0,000000
Treatment*Water volume	435,13	33	13,19	2,183	0,000872
Error	869,75	144	6,04		

Table 3.15 Anova table for deposition mortality, Nelspruit.

Univariate Tests of Significance for Mortality % 28 (Glufosinate 2 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 7.7108

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	118959ε	1	118959ε	20007,83	0,00
Treatment	126903	11	11537	194,03	0,00
Water volume	37043	3	12348	207,68	0,00
Treatment*Water volume	24423	33	740	12,45	0,00
Error	8562	144	59		

Table 3.16 Anova table for 80% mortality, Nelspruit.

Univariate Tests of Significance for Mortality days to 80% (Glufosinate 2 28 DAA in Deposition data 28 DAA 2019-08-04)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 2.4576

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	55658,13	1	55658,13	9215,028	0,000000
Treatment	5166,18	11	469,65	77,758	0,000000
Water volume	541,81	3	180,60	29,901	0,000000
Treatment*Water volume	435,13	33	13,19	2,183	0,000872
Error	869,75	144	6,04		

Table 3.17 Anova table for coverage, Nelspruit.

Univariate Tests of Significance for % Cover Glufosinate 1 (GLUFOSINATE in DATA Coverage 2019072
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 11.2045

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	69344,6	1	69344,6	552,368	0,00000
Treatment	3977,9	10	397,8	3,168	0,00114
Water volume	5087,7	3	1695,9	13,508	0,00000
Treatment*Water volume	10976,6	30	365,8	2,914	0,00001
Error	16571,3	132	125,5		

Table 3.18 Anova table for efficacy necrosis, Nelspruit.

Univariate Tests of Significance for Necrosis % 28 (Glufosinate 2 28 DAA in DATA Mortality 28 DAA data 20190806)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 5.1875

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	235900,5	1	235900,5	8766,368	0,00
Treatments	22005,7	11	2000,5	74,342	0,00
Error	968,8	36	26,9		

Table 3.19 Anova table for efficacy stunting, Nelspruit.

Univariate Tests of Significance for Stunting % 28 (Glufosinate 2 28 DAA in DATA Mortality 28 DAA data 20190806)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 14.0064

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	175208,3	1	175208,3	893,0973	0,000000
Treatments	19079,2	11	1734,5	8,8412	0,000000
Error	7062,5	36	196,2		

Table 3.20 Anova table for efficacy mortality, Nelspruit.

Univariate Tests of Significance for Mortality % 28 (Glufosinate 2 28 DAA in DATA Mortality 28 DAA data 20190806)
Sigma-restricted parameterization
Effective hypothesis decomposition; Std. Error of Estimate: 13.1630

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	335002,1	1	335002,1	1933,479	0,000000
Treatments	33160,4	11	3014,6	17,399	0,000000
Error	6237,5	36	173,3		