Chapter 7

Summary

7.1 Summary

Diuraphis noxia (Russian wheat aphid; RWA) feeding on wheat is considered to be a great pest in South Africa. Due to wheat being a major staple crop in South Africa and with RWA potentially causing 60% loss in wheat yield, studies have been conducted on obtaining wheat that is RWA resistant. Recent studies have concentrated on identifying the resistance that these plants pose as the possibility remains that the RWA could overcome this resistance.

In this thesis, various aspects of the factors affecting the resistance encountered by the RWA on wheat were investigated. In Chapter 3 it was found that RWA nymphs were found to prefer the susceptible wheat cultivars ('Palmiet' and 'Tugela') in that more nymphs survived to reach reproductive maturity compared to nymphs on the resistant wheat cultivar ('Tugela *Dn1*'). The RWA nymphs that survived to adulthood on the resistant cultivar showed no significant differences to those surviving on the susceptible cultivars with regard to reproduction and longevity. This is the first reported case that the surviving aphids show no statistical differences when comparing reproduction and life span on resistant and susceptible wheat plants.

In Chapter 4 an artificial diet was developed for the RWA. It was found that the RWA had dietary requirements for three important sources. Firstly, a carbon source (sucrose) with an optimum concentration of 20%, that allowed a greater number of aphids to survive and become reproductively active when transferred to the artificial diet. Secondly, the RWA showed a requirement for a nitrogen source (methionine, leucine and tryptophan). The addition of these three essential amino acids to 20% sucrose showed a significant increase in the average number

of nymphs being produced each day as compared to that of only the 20% sucrose solution. Thirdly, the addition of the two salts (magnesium chloride and potassium phosphate) resulted in an increased life span. In this chapter, a simple and effective diet was developed (Diet A) for the RWA. Although, it does not mimic the conditions encountered by the aphid in a wheat plant, it provides an easy and effective method of testing for the efficacy of potentially resistant compounds to the RWA.

In an investigation into the influence of RWA feeding on the protein profiles of RWA resistant wheat ('Tugela *Dn1*'), it was shown that there is an induction of seven proteins. There are two proteins absent after RWA feeding on 'Tugela *Dn1*'. These proteins occurred in both SDS-PAGE analysis and two-dimensional gel electrophoresis, with proteins of the same size (occurring in the same band on the SDS-PAGE gel) being separated according to their charge. Subsequently, the ≈ 20 kDa band observed with SDS-PAGE analysis, was revealed as three proteins upon two-dimensional gel electrophoresis with pI values of 5.0, 5.2 and 5.8. These induced proteins could not be sequenced due to their low concentrations in relation to the other proteins. Overexpression and underexpression of proteins were also visible after RWA infestation. Two induced proteins (≈ 36 and 26 kDa) are possibly β-1,3-glucanases due to their molecular size and p*I* values corresponding to previous studies done. The ≈ 20 kDa induced protein occurs closely to the third group of proteins described by Van der Westhuizen *et al.* (1998). Their function is unknown. The other unique proteins in this study have not been identified.

In Chapter 6 the leaf epicuticular wax ultrastructure and leaf trichomes were examined on two RWA susceptible wheat cultivars ('Palmiet' and 'Tugela') and a RWA resistant wheat cultivar ('Tugela *Dn1*') to determine what effect these might have on the RWA resistance. The lengths of the trichomes gave no significant differences for the three wheat cultivars examined. Trichome density showed that the resistant cultivar ('Tugela *Dn1*') had a significantly greater trichome density than the susceptible cultivars. 'Tugela' had a significantly lower trichome density than 'Palmiet', when comparing the two susceptible wheat cultivars. When examining the position of the trichomes it was revealed that there were differences for the adaxial and abaxial surfaces. Trichomes of the three wheat cultivars were found to occur mostly on the leaf veins of the adaxial surfaces whereas they were found to occur on the leaf veins as well as between them on the abaxial surfaces. Leaf trichome density acts as a physical obstacle to RWA feeding, as these mostly occur on the adaxial leaf veins were the RWA feeds. Subsequently, only the density of the trichomes seems to play a role as an obstacle to feeding by the RWA. Comparison of the SEM photos showed that the epicuticular wax structure was found to be similar amongst the three wheat cultivars studied for both the adaxial and abaxial surfaces. As the wax structure was similar on the RWA resistant and susceptible cultivars, the structure of the wax does not seem to play a role in RWA feeding.

Knowledge obtained from this thesis will hopefully be used in providing a better understanding of the insect-plant interaction. The exact resistance encountered by the RWA when feeding on a resistant plant does, however require more investigation. Studies aimed at identifying the proteins induced upon RWA infestation would enable a better understanding of the insect-plant interaction.

7.2 Opsomming

Die Russiese koringluis (*Diuraphis noxia*) is een van die ernstigste peste van koring in Suid-Afrika. Die Russiese koringluis kan tot 60% skade in koringproduksie veroorsaak en daarom is studies gedoen om Russiese koringluis-weerstandbiedende kultivars te vind. Onlangse studies het gefokus op die identifisering van die weerstand want die moontlikheid bestaan dat die Russiese koringluis hierdie weerstand kan oorkom.

In hierdie tesis word verskeie aspekte van die faktore bespreek wat die Russiese koringluis tydens voeding teëkom. In Hoofstuk 3 is bevind dat die Russiese koringluisnimf die vatbare koring kultivars ('Palmiet' en 'Tugela') bo die weerstandbiedende kultivar ('Tugela *Dn1*') verkies. Meer nimfe oorleef om reproduktief te word op vatbare kultivars in vergelyking met nimfe op die weerstandbiedende kultivar. Die Russiese koringluisnimfe wat tot volwassenheid op 'Tugela *Dn1*' bereik, toon geen betekenisvolle verskille in vergelyking met die nimfe wat op die vatbare kultivars oorleef ten opsigte van reproduksie en lewensduur nie. Hierdie is die eerste verslag waar die oorlewende nimfe geen betekenisvolle verskille toon as na die lewensduur en reproduksie op vatbare en weerstandbiedende koringplante gekyk word nie.

In Hoofstuk 4 is 'n kunsmatige dieet vir die Russiese koringluis ontwikkel. Daar is gevind dat die luis drie belangrike voedingsbronne vereis. Eerstens, 'n koolstofbron (sukrose), teen 'n optimale konsentrasie van 20%. Hierdie konsenstrasie laat meer luise tot volwassenheid oorleef om reproduktief te word na oordrag op die kunsmatige dieet. Tweedens, die Russiese koringluis het 'n vereiste vir 'n stikstofbron (metionien, leusien en triptofaan). Byvoeging van hierdie drie

essentiële aminosure tot die 20% sukrose toon 'n betekenisvolle verhoging in die reproduktiwiteit (gemiddelde getalle nimfe per dag) wanneer dit met die suiwer 20% sukrose-oplossing vergelyk word. Derdens, byvoeging van twee soute (magnesiumchloried en kaliumfosfaat) gee 'n verhoogde lewensduur. In hierdie hoofstuk is 'n eenvoudige en effektiewe kunsmatige dieet ontwikkel (Dieet A). Hoewel dit nie die toestande wanneer die luis op 'n plant voed naboots nie, voorsien hierdie kunsmatige dieet 'n eenvoudige en effektiewe metode om die doeltreffendheid van potensiële weerstandbiedende verbindings te toets.

In 'n ondersoek om vas te stel wat die invloed van Russiese koringluisinfestering op die proteïenprofiele van weerstandbiedende koring ('Tugela *Dn1*') is, is bevind dat daar 'n induksie van sewe proteïne is (Hoofstuk 5). Daar is twee proteïene afwesig na Russiese koringluisinfestering. Hierdie sewe proteïene word aangetref wanneer proteïene in beide SDS-PAGE-jels, sowel as twee-dimensionele jel-elektroforese geskei word. Proteïene wat in dieselfde band op die SDS-PAGE-jel voorkom, word volgens lading met twee-dimensionele jel-elektroforese geskei. Vervolgens, wanneer die ≈ 20 kDa band wat waargeneem word met SDS-PAGE-analise, geskei word met behulp van twee-dimensionele jel-elektroforese, is die produk drie proteïene met pI-punte 5.0, 5.2 en 5.8, onderskeidelik. Die indentiteit van die geïnduseerde proteïene kon nie bepaal word nie, as gevolg van hul lae konsentrasies. Daar was ook proteïene wat meer of minder uitgedruk is na Russiese koringluisinfestering. Twee van die geïnduseerde proteïene (≈ 36 en 26 kDa) is moontlik β-1,3-glukanase, aangesien hul pI-punte en grootte ooreenstem met vorige studies op proteïene. Die ≈ 20kDa geïnduseerde proteïen lê naby aan die derde groep proteïene wat deur Van der Westuizen *et al.* (1998) beskryf is. Hulle funksie is

onbekend. Die funksies van die ander unieke proteïene wat waargeneem was in hierdie studie is ook nie bekend nie.

In Hoofstuk 6 is die blaarepikutikulêre wasultrastruktuur en blaartrigome in twee Russiese koringluis vatbare koringkultivars ('Palmiet' en 'Tugela') en in 'n weerstandbiedende koringkultivar ('Tugela *Dn1*') ondersoek om die effek daarvan op Russiese koringluisweerstand vas te stel. Die lengte van die trigome het geen betekenisvolle verskil tussen die drie koringkultivars wat ondersoek is, getoon nie. Wanneer trigoomdigtheid ondersoek is, is getoon dat die weerstandbiedende koringkultivar ('Tugela *Dn1*'), betekenisvol meer trigome besit as die twee vatbare kultivars. Wanneer die twee vatbare kultivars vergelyk word, het 'Tugela' 'n betekenisvolle laer trigoomdigtheid as 'Palmiet'. Blaartrigome kan 'n fisiese hindernis wees wanneer die Russiese koringluis voed, want die trigome word op die blaarare waar die luis voed, aangetref. Vervolgens speel net die trigoomdigtheid 'n rol as 'n fisiese hindernis vir die Russiese koringluis. 'n Ondersoek na die posisie van die trigome op die blaaroppervlak het getoon dat daar verskille is in die abaksiale en adaksiale oppervlakte. Trigome van die drie koringkultivars het meestal op die beaarde adaksiale oppervlakte op en tussen die blaarare voorgekom. Vergelyking tussen SEM-fotos van die blaarepikutikulêre wasultrastruktuur het aangetoon dat dit eenders was vir die drie kultivars vir beide die adaksiale en abaksiale oppervlaktes. Dus speel die wasultrastruktuur waarskynlik nie 'n rol in Russiese koringluisweerstand nie.

Kennis vauit hierdie tesis verkry kan gebruik word om 'n beter begrip te bekom oor insek-plant interaksies. Die presiese weerstand wat die Russiese koringluis teëkom as hy voed op 'n weerstandbiedende plant vereis nog navorsing. Studies gemik op die identifiseering van die

geïnduseerde proteïene sal wetenskaplikes in staat stel om 'n beter begrip te hê van die insek-plant interaksie.