Genetic variability for Russian wheat aphid, *Diuraphis noxia* resistance in South African wheat genotypes

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Declaration

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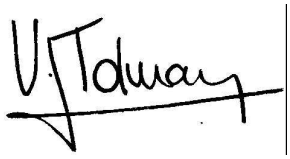

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Chapter 1
Introduction

Wheat as food crop in the world and South Africa.

Ninety-five per cent of the world's calories now come from only 30 crops, and 50% from just four: rice, maize, wheat and potato (Webb, 2000). Wheat is the most widely grown cereal crop in the world, and the world trade for wheat is greater than for all other crops combined (Curtis, 2002). Wheat originated in the Fertile Crescent of the Middle East from where it spread to North Africa, Eurasia, Western Europe, the Americas and the Southern hemisphere (Pagesse, 2000). Feldman (2000) describes the origin of cultivated wheats, which are divided into three main groups: diploids [2n=2x=14] (einkorn), tetraploids [2n=4x=28] (emmer, durum, rivet, Polish and Persian wheat) and hexaploids [2n=6x=42] (spelt, bread, club and Indian shot wheat). Hexaploid bread wheat, *Triticum aestivum*, presumably originated in northwestern Iran or northeastern Turkey as a result of a hybridisation between tetraploid wheat and diploid *Aegilops tauschii* some >8000 years BC. Due to the polyploid genetic structure of *Triticum* species and the associated genetic diversity, these plants have successfully been established throughout the world in varying environments.

The average global world wheat production from 1995-1999 was 584 million tons per annum (Maratheé and Gómez-MacPherson, 2000) and world production is expected to reach 860 million tons per annum by 2030. For the period 1995-1997 the average wheat consumption in the world was 73kg/person/year compared to that for sub-Saharan Africa in the same period of only 15kg/person/year. The per capita wheat consumption in South Africa is closer to the world average at 75.6kg/person/year (Payne, Wanjama and Girma, 2000). The annual wheat production in South Africa ranges from 1.7–2.7 million tons per annum (NDA, 2000) depending on the season and with an annual consumption of 2.8 million tons per annum South Africa is a net importer of wheat. Profit margins for producers are slim, with prices determined in a free market environment, linked to the international trade and influenced by the Rand/USD exchange rate.

Wheat is cultivated in three distinct production areas in South Africa. The mediterranean, winter rainfall region in the Western Cape grows dryland spring wheat and contributes approximately 30% of the annual yield while 20% of the annual yield is produced by irrigated spring wheat grown in the central irrigation areas including the Northern Cape. The remaining 50% of annual production is derived from dryland winter and intermediate/facultative wheat grown in the summer rainfall region on stored soil moisture that was accumulated during the preceding summer and autumn. This is a unique production system characterised by low seeding rates of 15-30 kg/ha using cultivars with long coleoptiles (>6cm) and a high tillering ability. Wheat is planted from May to August and harvested from late November to January depending on the season. The summer rainfall season stretches from October to February, but earlier spring rains can occur from August. Abiotic stress factors in this system include aluminium toxicity due to acid soils and pre-harvest sprouting due to rainfall during the harvest season while biotic stress factors include both diseases such as stripe

rust (*Puccinia* Westend *f. sp. striiformis* Eriks.), leaf rust (*Puccinia triticina* Eriks.*)*, take-all (*Gaeumannomyces graminis* var. *tritici*), glume blotch (*Septoria nodorum* Berk.) and crown rot (*Fusarium* spp) as well as a number of insect pests of which Russian wheat aphid, *Diuraphis noxia* (Kurdjumov), is the most important.

Russian wheat aphid, Diuraphis noxia (Kurdjumov) (Homoptera: Aphididae)

D. noxia is a small (<2.0 mm), spindle shaped, pale yellow-green to grey-green aphid with extremely short antennae (Figure 1.1). The dorsal process of the 8th abdominal tergite gives the impression of a "double tail" when viewed laterally and the siphunculi are not prominent (Du Toit and Aalbersberg, 1980; Walters, Penn, Du Toit, Botha, Aalbersberg, Hewitt and Broodryk, 1980). Reviews and bibliographies of the Russian wheat aphid have been published by Hughes (1988), Kovalev, Poprawski, Stekolshchikov, Vereshchagina and Gandrabur (1991) and Poprawski, Underwood, Mercadier and Gruber (1992).

FIGURE 1.1: Apterous Russian wheat aphid [Photograph:J.L. Hatting]

Biology

D. noxia has four nymphal instars and an adult stage. A simple key for the diagnosis of the instars, using morphology of the antennae, caudae and wing buds in conjunction with ratios between antennal segment lengths, was developed by Aalbersberg, Van der Westhuizen and Hewitt (1987). As far as is known, only female *D. noxia* occur in South Africa and reproduction is parthenogenetic with both the alate and apterous forms of *D. noxia* being reproductive. Alate formation occurs when the host plant is under stress or when the host plant no longer provides a favourable habitat (Walters *et al*., 1980). *D. noxia* is able to survive temperatures as low as –20 ℃ (Butts, 1992). Nymph production is optimal at temperatures higher than 5 ℃ and lower than 20 ℃ and can peak at 4 nymphs per day with a total of 70 nymphs produced per female in a typical lifetime (Robinson, 1992).

D. noxia is known to reproduce sexually in other parts of the world. Kiriac, Gruber, Poprawski, Halbert and Elberson (1990) reported on the occurrence of sexual morphs (both oviparae and males) of Russian wheat aphid in several locations in the Soviet Union but only the presence of oviparae in Idaho and Oregon in the USA, speculating that North American *D. noxia* my be gynocyclic. Basky (1993b) reported *D. noxia* in Hungary to be holocyclic. Clúa, Castro, Ramos, Giménez, Vasicek, Chidichimo and Dixon (2004) reported that only 20% of the 22 *D. noxia* clones collected throughout Argentina and Chile produced sexuals irrespective of the host they were collected from, the period of the year, region, current host, day length and average temperature of the rearing conditions.

Distribution and Biotypes

D. noxia is endemic to central Asia, southern Russia, countries bordering the Mediterranean Sea, Iran and Afghanistan (Durr, 1983; Hewitt, Van Niekerk, Walters, Kriel and Fouchè, 1984; Dolatii, Ghareyazie, Moharramipour and Noori-Daloii, 2005) but now occurs in virtually all the major small grain production regions of the world except northeastern China (Robinson, 1992) and Australia (Hughes and Maywald, 1990) where it is listed in the Grainguard Threat Data Sheet for the wheat Industry (Botha and Hardie, 2000).

The earliest published reference to *D. noxia* as a pest was in the Crimea (Mokrzhetsky, 1901 as quoted by Kovalev *et al*., 1991). Sporadic outbreaks of this pest have occurred in the former USSR since, with losses of 75% reported due to infestations of this aphid in 1912 (Mokrzhetski (1914) as cited by Halbert and Stoetzel, 1998). More recently damage caused by *D. noxia* was restricted to the steppe zone of the Ukraine and Russian Soviet Federated Socialist Republic (Voronin, Shapiro and Pukinskaya, 1988 as quoted by Kovalev *et al*., 1991). An epidemic was reported in 1962 in the Konya Province in Turkey where crop losses of 25-50% occurred (Elmali, 1998). In Africa *D. noxia* was reported in the Wukro (Atsbi) and Adigrat regions of Ethiopia in 1972/73 and from the western Welo region in 1974. By 1976 *D. noxia* was widespread in all the barley and wheat growing areas of Ethiopia (Haile, 1981) and was considered to be the leading pest of cereals in the highlands of Ethiopia. Barley grain yield losses of 41-71% were reported in Ethiopia by Miller and Adugna (1998). In South Africa, *D. noxia* was first reported as a pest of wheat in 1978 (Walters, 1984) and has occurred annually since. Attia and El-Kady (1988) observed *D. noxia* on wheat and barley in Beni-Suef Province in Egypt during 1985, noting subsequent spread to other cereal producing areas of Egypt. In 1995 *D. noxia* was also reported in Kenya where yield losses of 25-90% occurred (Kiplagat, 2005). In 1980 this pest was found to be present in Mexico (Gilchrist, Rodriguez and Burnett, 1984), the first report of its presence on the American continents. By 1986 it was reported to be present in Texas and is now found in 17 western states of the United States (Miller, Porter, Burd, Mornhinweg and Burton, 1994). In July 1988, *D. noxia* was detected in Canada. It was first recorded in southern Alberta and spread to Saskatchewan and British Columbia by the end of the year (Jones, Byers, Butts and Harris, 1989). Russian wheat aphid has been reported in Chile and Argentina where it was initially reported in 1988 and 1992 respectively (Ortego and Delfino, 1994 as cited by Clúa *et al*., 2004). It was found in the main cereal-producing region of Argentina in 1994 (Bellone and Amaraz,

1995 as cited by Clúa *et al*., 2004) and then spread northwards and eastwards infesting *T. aestivum* and *T. durum* in 1995 (Castro, Ramos, Vasicek, Worland, Giménez, Clúa and Suárez, 2001). In Europe, *D. noxia* is also known to occur in Hungary (Basky, 1993b) although it is not an economically significant pest there (Basky, 1993a; Tolmay, Basky and Lang, 2001), Serbia (Petrović, 1992 as cited by Starý, Basky, Tanigoshi and Tomanovicć, 2005), Slovakia (Lukáš, Toth, Vráblová, Lukášová and Cagán, 1999 as cited by Starý *et al*., 2005), Croatia (Barčič and Čuljak, 2002 as cited by Starý *et al*., 2005), Romania (Holman and Pintera, 1981 as cited by Starý *et al*., 2005) and Austria (Cate, 2000 as cited by Starý *et al*., 2005). Zhang (1991) as cited by Botha and Hardie (2000) reported that *D. noxia* had been known to occur in the Xinjiang-Uiger Autonomous Region of the Peoples Republic of China for decades, but that it had not spread to the major wheat growing areas of central China.

Several studies have indicated the presence of diversity in *D. noxia* populations found in various parts of the world. Puterka, Burd and Burton (1992) have shown that *D. noxia* from different parts of the world vary in their reaction to resistant wheat lines in the USA. Puterka, Black, Steiner and Burton (1993) found strong similarities between United States populations of *D. noxia* and collections from South Africa, Mexico, France and Turkey with most variation detected among populations from the Middle East and southern Russia. Differences have been reported between *D. noxia* in South Africa and Syria (Black, DuTeau, Puterka, Nechols and Pettorini, 1992), as well as between South Africa and Hungary (Basky, Hopper, Jordaan and Saayman, 2001). Black *et al.* (1992) amplified DNA from individual *D. noxia* nymphs and adults collected from South Africa, and found that there appeared to be two genotypic patterns in the South African Russian wheat aphid population while the Syrian population appeared homogenous.

In the USA, a study by Shufran, Burd and Webster (1997) reported baseline information on the biotipic status of *D. noxia* prior to the commercial planting of resistant cultivars indicating no genotypic variation in aphid clones collected from various localities on barley and wheat. The detection of a new biotype of *D. noxia* in Colorado in 2003, which is virulent to commercially resistant cultivars containing the *Dn4* resistance gene (Haley, Peairs, Walker, Rudolph and Randolph, 2004) sparked renewed interest in studying biotypes of *D. noxia* throughout the world. Belay, Smith and Stauffer (2004) reported finding no biotypic variation within Ethiopian *D. noxia* based on damage ratings of various resistant lines, however the Ethiopian, Czech and Chilean biotypes of *D. noxia* were all virulent to *Dn4* (Smith, Belay, Stauffer, Starý, Kubeckova and Starkey, 2004). The genetic marker study linked to this work was not successful in detecting significant variation in polymorphisms to detect biotypic variation. Dolatti *et al*. (2005) studied the regional diversity and host adaptaion of Iranian *D. noxia* populations finding that one or a few widespread genotypes occurred along with many rare genotypes. Differentiation was also observed between *D. noxia* collected off barley and wheat. *D. noxia* is native to Iran and the high genetic diversity reported by this study can be explained by the possibility of sexual reproduction of the aphid in this region as well as the long period of time that the aphid has been present in the area. The presence of a resistance breaking bioype of *D. noxia* in South Africa was confirmed in December 2005 (Tolmay, Lindeque and Prinsloo, 2006). All

cultivars marketed as resistant during the 2005 season are severely damaged by the new biotype, which has not yet been characterised against the international differential set of resistance genes.

Damage

Yield losses due to *D. noxia* are severe with individual plant losses as high as 90% possible (Du Toit and Walters, 1984). Robinson (1992) recorded crop losses of 68% in Ethiopia and 35-60% in South Africa for wheat. Yield losses in Ethiopia for barley were estimated to be between 41-71%. In 1993 the yield losses caused by *D. noxia* in South Africa amounted to approximately R30 million (Swart, 1999) with approximately R15 million spent on chemical control annually (Cilliers, Tolmay and Van Niekerk, 1992). In the United States losses due to *D. noxia* have been quantified as running into millions of dollars annually. The cumulative economic loss (1987-1993) attributed to *D. noxia* in the United States exceeds $890 million, with approximately $83 million being spent on control, $349 million in lost production and $460 million in additional lost economic activity in local communities (Webster and Amosson, 1994).

The symptoms of *D. noxia* infestation are very distinct. Typical white, yellow and purple to reddish-purple longitudinal streaks occur on the leaves of plants infested with *D. noxia*. The aphids are found mainly on the adaxial surface of the newest growth, in the axils of leaves or within rolled leaves. Heavy infestations in young plants cause the tillers to become prostrate, while heavy infestations in later growth stages cause the ears to become trapped in the rolled flag leaf (Walters *et al*., 1980). Severe damage is associated with these symptoms. The toxin or biochemical reaction that causes the damage has not yet been identified, though the effects are well known. *D. noxia* infestation leads to a drastic reduction in chlorophyll content (Kruger and Hewitt, 1984) and reduced photosynthetic ability (Fouché, Verhoeven, Hewitt, Walters, Kriel and de Jager, 1984) which, when combined with the characteristic leaf rolling that occurs, causes a considerable loss of effective leaf area of susceptible plants (Walters *et al*., 1980). Matsiliza (2003) showed that *D. noxia* feed preferentially from thin-walled sieve tubes in sink as well as source leaves of wheat and that the small longitudinal bundles were preferred. Eighty three percent of stylet tracks in sink leaf material terminated in thin-walled sieve tubes while on source leaf tissue 95% of stylet tracks also terminated in thin-walled sieve tubes. It was postulated that the preference for these veins is likely to be related to the quality and quantity of assimilates in them as these veins have been implicated in the assimilate loading in source leaves. By feeding on minor rather than major veins the aphid has the advantage of a shorter pathway to the sieve tubes, less sclerenchyma to impede the passage of the stylets and a food source which may be richer in both sugars and proteins as the smaller veins have been implicated in the loading and unloading of assimilates. Using analine blue stain Botha and Matsiliza (2004) reported that *D. noxia* infested leaf tissue (wheat cv Adamtas) was heavily callosed, with callose deposited between the plasma membrane and the cell wall, not only within the phloem tissue, but also in neighbouring vascular parenchymea cells. Deposition of wound callose was found to have disrupted phloem transport and thus the export of photo-assimilate from

the leaves. Matsiliza (2003) confirmed that typical of most aphids, *D. noxia* probes the leaf between epidermal cells or through the stomata and proceeds on an intercellular pathway through the mesophyll cells to the vascular tissue from where pathway is intracellular, near and inside the bundle and that feeding aphids form local sinks, once their stylets have penetrated the functional phloem. Burd and Burton (1992) showed that *D. noxia* infestation resulted in water imbalances in the host plant, expressed as a loss of turgor and reduced growth. Substantial reductions in plant biomass also occur (Burd and Burton, 1992).

Many factors influence *D. noxia* damage. It is widely accepted that *D. noxia* show a preference for stressed host plants and that plants grown under drought stress or low nitrogen levels are more damaged. Johnson, Ni, McLendon, Jacobsen and Wraith (1998) reported that drought stressed wheat plants infested with *D. noxia* showed higher leaf surface temperatures and argued that *D. noxia* inside a longitudinally rolled leaf could maintain higher body temperatures and may thus attain maximal developmental and reproductive rates. The level of infestation, the growth stage of the host plant and the duration of the infestation all influence the severity of the damage caused by *D. noxia.* Du Toit and Walters (1984) concluded that wheat plants were most sensitive to *D. noxia* infestation from the flag leaf stage to flower initiation. Burd and Burton (1992) indicated that the duration of infestation, rather than the level of infestation may be more important when damage is caused to the host plant. In colder climates reduced coldhardiness and therefore also plant survival and yield due to *D. noxia* infestation were reported by Storlie, Talbert, Taylor, Ferguson and Brown (1993). This was found to be associated with higher osmotic potentials and lower fructan content in winter wheat infested with *D. noxia.*

Phloem feeding insects are well known for their ability to transmit plant viruses. *D. noxia* was reported as a vector of barley yellow dwarf virus, brome mosaic virus and barley stripe mosaic virus (Von Wechmar, 1984). Cronjè (1990) found that *D. noxia* in South Africa, was not an effective vector of brome mosaic virus, with only 20 percent successful transmission under controlled conditions. Researchers in the United States have been unable to confirm any significant transmission of viruses by *D. noxia* (Damsteegt, Gildow, Hewings and Carroll, 1992; Halbert, Connelly, Bishop and Blackmer, 1992).

Chemical control measures

The effective control of Russian wheat aphid has been a significant challenge facing wheat producers and researchers alike throughout the regions where this pest occurs. Due to the aphids' habit of feeding within the rolled leaf whorl, options for chemical control of *D. noxia* have been limited to the use of systemic insecticides such as disulfoton, dimethoate and demeton-s-methyl, vapour action insecticides such as chlorpyriphos and parathion which can penetrate the rolled leaf and more recently seed dressings such as imidacloprid and thiametoxam (Nel, Crause and Khelawanlall, 2002). In many countries the use of some of the aforementioned insecticides was

discontinued due to environmental and safety concerns leaving very few chemical control options available to producers.

Non-chemical control measures

In South Africa damage to wheat crops can be limited by the use of systemic insecticides, but the large-scale use of insecticides has been discontinued as farmers are now planting resistant cultivars to control this pest (Tolmay, Prinsloo and Hatting, 2000) as a key component of an integrated control strategy against *D. noxia* in both commercial and small-scale production situations. World-wide the use of insect-resistant cultivars is seen as one of the most desirable alternatives to insecticides because of their low cost and environmentally friendly action (Burton, Porter, Baker, Webster, Burd and Puterka, 1991; Quisenberry and Schotzko, 1994). Resistance breeding against *D. noxia* takes place in South Africa (Tolmay and Van Deventer, 2005; Van Niekerk, 2001), the USA (Quick, Ellis, Normann, Stromberger, Shanahan, Peairs, Rudolph, and Lorenz, 1996), at CIMMYT in Mexico and ICARDA in Syria (Robinson, 1992) and pre-emptively in Australia (Botha and Hardie, 2000).

Plant defence and host plant resistance

Host plant resistance to insect pests of crop plants is generally seen as an effective, environmentally responsible, economically and socially acceptable method of pest control which plays an integral role in sustainable agricultural systems (Wiseman, 1999). Pest resistant crops offer a solution that can be tailored to meet the specific need of producers while usually offering more benefits than drawbacks for the environment. The most important benefit of a pest resistant crop is the fact that the pest control occurs independently of the managerial ability, skill and resource level of the producer (Tolmay, 2001). Host plant resistance has been used as a control measure for various agricultural pests for many years (Smith, 1989).

Painter (1951) explained host plant resistance by using three functional categories, namely antibiosis, non-preference (antixenosis) and tolerance which describe the pest-host interaction. Antibiosis describes the negative influence of the plant on the biology of an insect attempting to use that plant as host (Smith, 1989). This may be expressed as reduced body size and mass, prolonged periods of development in the immature stages, reduced fecundity or failure to pupate or eclose. Antixenosis, the inability of a plant to serve as a host, is caused by physical or chemical plant factors that repel or deter insects from feeding or oviposition (Smith, 1989). Tolerance indicates the plant's ability to withstand or compensate for insect damage (Smith, 1989). Known components of this form of resistance include general vigour, compensatory growth, wound healing, mechanical support in tissues and organs and changes in photosynthetic partitioning. Environmental factors, however may affect tolerance more than other types of resistance (Pedigo, 1989). The mechanism of resistance in a specific line will influence the efficacy of the line in

controlling field populations of the pest and may in part determine the longevity of the resistance under field conditions by influencing the formation of a biotype (Gallun, 1972). Many factors play a role in the expression of host plant resistance and its effect on the target pest when deployed in resistant cultivars in the field.

The actual nature of the resistance within the plant itself has also been studied extensively (Agrawal, Tuzen and Bent, 2000). Gatehouse (2002) defines constitutive resistance as those morphological and chemical factors that are present in a plant prior to attack also known as passive defence, in contrast to induced resistance, which is defined as an active response by the plant to attack. Morphological factors include general tissue toughness, silica, calcium carbonate or lignin surrounding vascular bundles, leaf hairs and epicuticular wax, which form physical barriers to attack. Chemical factors are plant products that have some antimicrobial or antiherbivore (deter, poison, starve) activity and are often due to phenolics, alkaloids and proteins (Van der Westhuizen, 2004). Most protein-based defences known to date have an anti-nutritive effect on herbivores, destroying or preventing the assimilation of nutrients by the insect, thereby slowing its growth and development (Constabel, 2000). Overall, in terms of chemical factors, there is often no inherent difference between the chemistry of constitutive and induced defences with the accumulation / up-regulation pre-existing compounds being induced by herbivore damage.

It is generally accepted that the expression of constitutive resistance in plants is associated with a fitness cost that accrues when pests are absent and the magnitude of these costs is thought to explain why susceptible genotypes persist in plant populations (Cipollini, Purrington and Bergelson, 2003). Induced defences are only produced by plants under attack from pests. This defence can be localised, or systemic. Induced responses in plants to herbivore attack are thought to be a form of adaptive phenotypic plasticity, saving metabolic costs by expressing defences only when necessary (Cipollini *et al*., 2003). The costs of induced resistance responses have been shown to accrue as a result of the allocation of resources towards defence production and away from primary metabolism or even as a result of auto toxicity of defence chemicals. Ecological costs of induced resistance may include increased susceptibility to untargeted herbivores as shown by Agrawal, Gorski and Tallamy (1999) where increased levels of cucurbitacins in cucumber plants provided resistance to generalist arthropod herbivores while acting as a feeding stimulant for specialist beetles. In radishes there is evidence that increased resistance to herbivores may reduce the attractiveness of plants to pollinators (Karban and Nagasaka, 2004). The basic response of plants to herbivory is the wounding response, which is both local and systemic and usually involves multiple signalling pathways. Insects that feed on the content of the vascular tissue and avoid extensive tissue damage evade the wounding response and have been reported to activate the same defence response as pathogens (Gatehouse, 2002; Kaloshian and Walling, 2005).

In most cases of defences induced by insect herbivory, for both the wounding and pathogenesis pathways, saliva plays an important role in the elicitation of plant defense responses (Felton and

Eichenseer, 2000). Insect saliva performs a multitude of functions amongst others digestion, lubrication of the mouthparts, pH regulation and in some cases suppression of host response. Aphids secrete two types of saliva (Miles, 1959); one that gels soon after secretion, forming a sheath around the stylets and the other a 'watery saliva' which is secreted during ingestion. The stylet sheath is though to assist aphids by holding the stylets in place while probing, sealing wounds and fluid loss when individual cells are punctured, preventing ingestion of unacceptable fluids, preventing signals produced by aphid feeding from diffusing out of the wound area and adsorbing antifeedant phenolics (Felton and Eichenseer, 2000). A comprehensive paper on the saliva of Hemiptera was published by Miles (1972).

In order to effectively utilise plant defense mechanisms against pests in a breeding programme a comprehensive understanding of the mechanisms that underlie resistance responses is needed (Van der Westhuizen, 2004). Despite the many advantages of exploiting naturally occurring plant defences there are numerous challenges associated with this practice; combining resistance with high yield and good quality being one of the most important (Prado, 1997; Tolmay, 2001; Van der Westhuizen, 2004). Plant defence and the expression of resistance are affected by various factors including nutrient availability (Glynn, Herms, Egawa, Hansen and Mattson, 2003) and general physiological condition of the plant. Furthermore, though effective, resistance may not necessarily limit pest outbreaks. Morris and Dwyer (1997) have shown that constitutive resistance influences the speed of a herbivore invasion by influencing the spatial dynamics of herbivore populations most while both constitutive and inducible resistance alter demographically important rates of herbivore birth, growth and survival. Furthermore, it was demonstrated that if levels of constitutive resistance are high and herbivore movement is sensitive to host quality, the rate of herbivore spread could in effect be accelerated even though the intrinsic rate of increase is reduced.

Russian wheat aphid resistance

Since the first report of host plant resistance to *D. noxia* in *Triticum monococcum* (Einkorn), line A 544, and other *T. monococcum/T. durum* amphiploids (Du Toit and Van Niekerk, 1985) many other sources of resistance have been described. Though not exhaustive as others have been reported since then, the most comprehensive review of *D. noxia* resistance sources was compiled by Souza (1998) who listed 98 accessions of *Triticum aestivum* and related species reported resistant to *D. noxia* by various authors. Besides that reported in bread wheat, *D. noxia* resistance has been reported in *T. monococcum*, *T. turgidum*, *T. dicoccum*, *Secale cereale*, X *Tritcosecale*, *T. tauchii*, *Hordeum vulgare*, *H. bulbosum*, *H. bogdani* and *H. brevisubulatum.* Ten resistance genes have been identified and their chromosome location determined. Most *D. noxia* resistance genes identified to date are either located to the D chromosomes or to the rye translocation of wheat (Lage, Skovmand and Andersen, 2004). Seven of these genes namely *Dn1* (Marais and Du Toit, 1993; Schroeder-Teeter, Zemetra, Schotzko, Smith and Rafi, 1994), *Dn2* (Ma, Saidi, Quick and Lapitan, 1998), *Dn5* (Marais and du Toit, 1993), *Dn6* (Lui, Smith and Gill, 2002), as well as *Dn8*,

Dn9 and *Dnx* (Lui, Smith, Gill and Tolmay, 2001) have been located on the 7D chromosome of wheat. *Dn4* (Ma *et al*., 1998) was located to the 1D chromosome of wheat while *dn3* was found in a diploid D-genome *Aegilops tauchii* line. Marais, Wessels, Horn and Du Toit (1998) reported *Dn7* on a 1BL/1RS translocation from rye, *Secale cereale*. Efforts to broaden the genetic base for resistance have been attempted with *D. noxia* resistant intergeneric hybrids developed by Aung (1991) from crosses between *Hordeum vulgare* and *Elymus trachycaulus* and more recently with resistant synthetic hexaploid wheat developed from interspecific crosses of *Triticum dicoccum* and *Aegilops tauchii* where the resistance gene(s) have been shown to be located on the A and/or B genomes, therefore presumed different to previously identified resistance genes (Lage *et al*., 2004). *D. noxia* resistance identified in bread wheat has, however been the most deployed in breeding programmes to date.

In South Africa, the use of *D. noxia* resistant cultivars was made possible through the discovery of host plant resistance against this pest, in bread wheat, by Du Toit (1987; 1988; 1992). The first crosses between the resistance donors and adapted South African bread wheat cultivars were made in mid 1986, the first field evaluations of back-cross progeny were undertaken in 1989 (Du Toit, 1993) and the first cultivar, Tugela-Dn, released in 1992 (Van Niekerk, 2001). *D. noxia* resistant cultivars released for commercial use in South Africa have been shown to have a yield advantage above susceptible cultivars in farmers fields (Marasas, Anandajayasekeram, Tolmay, Martella, Purchase and Prinsloo, 1997). The adoption of *D. noxia* resistant cultivars in South Africa as documented by Marasas *et al*. (1997) was found to be limited only by the availability of seed. These cultivars provided a welcome alternative as the cost of systemic insecticides became prohibitive especially where harsh climatic conditions reduced their efficacy (Du Toit, 1988; 1992). It was estimated that between 70 and 85% of the area planted to wheat in 2001 was under resistant cultivars. These cultivars saved wheat producers approximately ZAR 120.00 (one tenth of the income per ton) per hectare by eliminating the need for chemical control making it easier to produce wheat at the same price it would cost to import wheat bought on the global market (Tolmay, 2001). The benefit was noticed in the environment as well; all insecticides, except two namely imidacloprid and amethoxam, registered for the control of Russian wheat aphid in South Africa were broad-spectrum systemic or contact organophosphates (LD_{50} 2-70mg/kg). Due to the rapid adoption of resistant cultivars the average area treated with insecticides decreased from 85% in 1990 to 30% in 1997 (Marasas, 1999). By 2006 a total of 27 cultivars with *D. noxia* resistance had been released in South Africa namely Betta-Dn, Caledon, Elands, Gariep, Komati, Limpopo, Matlabas, Nossob, PAN 3235, PAN 3364, PAN 3144, SST 124, SST 322, SST 333, SST 334, SST 347, SST 363, SST 367, SST 399, SST 935, SST 936, SST 946, SST 966, SST 972, SST 983, Tarka and Tugela-Dn (Dr A. Barnard, personal communication)[1].

The international trend for reducing the impact of *D. noxia* on small grains is the use of Russian wheat aphid resistant cultivars (Webster, Starks and Burton, 1987; Du Toit, 1989b; Robinson,

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Delgado, Vivar and Burnett, 1992). To date resistant cultivars have been developed and released not only in South Africa (Tolmay and Van Deventer, 2005) but in the USA in Colorado (Randolph, Peairs, Koch, Walker, Stubbs, Quick and Haley, 2005) and Kansas (Qureshi, Jyoti and Michaud, 2005). This proved to be a worthwhile investment with researchers in the USA estimating the return on investment in developing the resistant cultivar Halt to be 13:1 (Webster and Kenkel, 1999) while Marasas *et al.* (1997) reported a Rate Of Return benefit to society from the yield gains of resistant cultivars in South Africa at 34%.

Du Toit (1989a) reported the resistance in PI 137739 and PI 260660 to *D. noxia* to be governed by single dominant genes, which probably differ from each other. These genes were designated Dn1 and Dn2 respectively and the mechanisms of resistance in the donor lines shown to be antibiosis and antixenosis (Du Toit, 1987, 1989b). Various other authors also studied these original sources of *D. noxia* resistance, PI 137739 and PI 262660. Smith, Schotzko, Zemetra and Souza (1992) evaluated these lines under greenhouse conditions in Idaho to determine the categories of resistance. Based on percentage reduction in plant height it was concluded that both lines possessed a significant level of tolerance to *D. noxia* feeding. *D. noxia* maintained on these lines displayed reduced reproductive rates 21 days after infestation, indicating the presence of low-level antibiosis. *D. noxia* on PI 137739 was found to have a significantly lower reproduction rate than on PI 262660 and Stephens, the susceptible control, in a trial conducted by Quisenberry and Schotzko (1994). This indicated that PI 137739 showed antibiosis in contrast to PI 262660 which had higher plant growth, dry weight and moisture while expressing higher leaf chlorosis and mid-leaf rolling indicating tolerance. Mowry (1994) found that antibiosis in the lines PI 137739 and PI 262660 could not be detected statistically when uninfested plants were compared to susceptible controls, but that *D. noxia* performance was significantly less on these lines when Barley Yellow Dwarf Virus (BYDV) - infested plants were compared, indicating that plant stress influences the expression of *D. noxia* antibiosis.

A large number of studies have been conducted on various aspects of the biochemical and physical characteristics of *D. noxia* resistance. Both donor accessions and improved lines have been studied. Studies conducted on donor accessions are listed in Table 1.1. Regarding improved lines, various authors have studied two *D. noxia* resistant cultivars namely TugelaDn and Halt, developed in South Africa and Colorado (USA) respectively and their near-isogenic counterparts. A substantial amount of information is known regarding the nature and effect of the resistance in these two genotypes. TugelaDn [PI 591932] with resistance *ex* PI 137739 (Van Niekerk, 2001; Tolmay, Du Toit and Smith, 2006) and Halt [PI 584505] with resistance *ex* PI 372129 (Quick *et al.,* 1996), are accepted as containing the *D. noxia* resistance genes Dn1 and Dn4 respectively. The studies conducted using these lines have been listed in Table 1.2.

Available information suggests that *D. noxia* resistance includes both constitutive and induced elements with induced responses being the more important component. Examination of trichome presence and leaf epicuticular wax ultrastructure by Bahlmann, Govender and Botha (2003)

showed that susceptible Tugela had 1.7 times fewer trichomes per mm^2 (=16.39) than resistant Tugela-Dn (=28), while there was no difference in trichome length and epicuticular wax ultrastructure between the cultivars. Ni and Quisenberry (1997b) also reported a high density (=21.33 trichomes.mm^{-2}) of short trichomes (68.1 µm) on the adaxial leaf surface of the resistant cultivar Halt. Furthermore, a study on the distribution of *D. noxia* salivary sheaths on resistant Halt and susceptible Arapahoe wheat by Ni and Quisenberry (1997a) showed that the majority of sheaths were made by intercellular penetration of leaf epidermal cells on both cultivars but significantly more sheaths were made through leaf stomata on Halt than on Arapahoe possibly eluding to a need for easier access on the resistant host. Most sheaths terminated in the vascular bundles on both cultivars, with no significant difference being recorded between the cultivars.

In terms of induced defences, studies conducted regarding the biochemistry of *D. noxia* resistance indicate that the response is not a wounding response usually characteristic of herbivore damage, but a typical hypersensitive response (HR) more characteristic of pathogenesis (Van der Westhuizen, 2005). *D. noxia* infestation induced enhanced expression/synthesis of two polypeptides (100 kD [nuclear encoded] and 56 kD [organel encoded]) in the resistant Tugela-Dn and a decrease in the synthesis of a 45 kD polypeptide in both resistant Tugela-Dn and susceptible Tugela. Available evidence, molecular mass and high content suggest that the 56 kD protein is Rubisco (Van der Westhuizen and Botha, 1993). Studying susceptible Tugela and the near-isogenic resistant cultivar Tugela-DN, Van Der Westhuizen and Pretorius (1995) concluded that changes in the chlorophyll, protein, free amino acid, proline levels and respiration rate in response to *D. noxia* infestation indicate that a stress condition is induced in both susceptible and resistant wheat plants by *D. noxia* feeding. The unique changes in resistant wheat, especially the marked increase in the total free proline content, seems to contribute to the plants improved ability to cope with *D. noxia* infestation and therefore survive. Proline is known to play a protective role for membrane systems under stress; thus, membranes in resistant plants remain intact and photosynthesis can proceed relatively normally as opposed to susceptible plants where the chloroplasts are damaged. Although an increase in the total phenolic content in infested resistant plants may contribute a possible deterrent effect against *D. noxia,* none of the other observed biochemical changes in resistant wheat could be regarded as detrimental to *D. noxia*.

TABLE 1.1: List of studies conducted with *D. noxia* resistant donor accessions

Accession and Topic of Study	Reference
PI 137739	
Components of resistance	Du Toit (1989b)
Population development and plant damage	Quisenberry and Schotzko (1994)
Reproductive rate and population development	Rafi, Zemetra and Quisenberry (1996)
Feeding damage	Rafi, Zemetra and Quisenberry (1997)
DIMBOA concentration	Ni and Quisenberry (2000)
PI 140207	
Reproductive rate and population development	Rafi *et al.* (1996)
PI 262660	
Components of resistance	Du Toit (1989b)
Population development and plant damage	Quisenberry and Schotzko (1994)
Reproductive rate and population development	Rafi *et al.* (1996)
DIMBOA concentration	Ni and Quisenberry (2000)
PI 294994	
Components of resistance	Du Toit (1989b)
DIMBOA concentration	Ni and Quisenberry (2000)
PI 372129	
Winterkill, osmotic potential and fructan content	Storlie *et al.* (1993)

Furthermore the resistance in TugelaDn is associated with elicitor-active, intercellular, infestation-related glycoproteins in the 28-33 kDa range (Van der Westhuizen and Pretorius, 1996). *D. noxia* infestation dramatically changed intercellular protein composition of resistant wheat with differential induction of β-1,3-glucanase (Van der Westhuizen *et al.*, 1998a), chitinase and peroxidase (Van der Westhuizen *et al.*, 1998b) while in the absence of *D. noxia* the apoplastic fluid of resistant and susceptible near-isolines was similar. These enzymes, also known as PR-proteins, were induced systemically and are known to be associated with plant defence against invading pathogens. More detailed studies of β-1,3-glucanase *in planta* using an immunogold labelling technique (Van der Westhuizen *et al.*, 2002) showed that β-1,3-glucanase accumulated in tissues of resistant wheat most affected by aphid feeding, in particular the cell walls of vascular bundle cells and the chloroplasts. PR-protein activity in resistant wheat has also been shown by Ni *et al.* (2001) who reported that *D. noxia* feeding elicited a moderate increase (approximately threefold) of peroxidase specific activity in Halt which contributed to the resistance of this cultivar.

TABLE 1.2: List of studies conducted with *D. noxia* resistant cultivars TugelaDn and Halt

Cultivar and Topic of Study	Reference
TugelaDn	
Composition and synthesis of water soluble proteins	Van der Westhuizen and Botha (1993)
Free proline, total phenolic content and respiration rate	Van der Westhuizen and Pretorius (1995)
Protien composition of apoplastic fluid	Van der Westhuizen and Pretorius (1996)
Apoplastic peroxidase and chitinase activities	Van der Westhuizen, Qian and Botha (1998b)
ß-1-3-glucanase activity	Van der Westhuizen, Qian and Botha (1998a)
Expression of chitinase isoenzymes	Botha, Nagel, van der Westhuizen and Botha (1998)
Purification and localisation of ß-1-3-glucanase induced by *D. noxia* feeding	Van der Westhuizen, Qian, Wilding and Botha (2002)
Salicylic acid in the resistance response of wheat to *D. noxia*	Mohase and van der Westhuizen (2002)
Trichome presence and leaf epiculticular wax ultrastructure	Bahlmann *et al.* (2003)
Enzymatic chlorophyll degradation	Wang, Quisenberry, Ni and Tolmay (2004b)
Photosynthetic pigment concentrations and chlorphyll / carotenoid ratios	Wang, Quisenberry, Ni and Tolmay (2004a)
Halt	
Distribution of *D. noxia* salivary sheaths	Ni and Quisenberry (1997a)
Leaf epicuticular structure	Ni and Quisenberry (1997b)
Influence of epicuticular wax on probing and nymphoposition	Ni, Quisenberry, Siegfried and Lee (1998)
Phloem composition	Telang, Sandström, Dyerson and Moran (1999)
Oxidative response to *D. noxia* feeding	Ni, Quisenberry, Heng-Moss, Markwell, Sarath, Klucas and Baxendale (2001)
Plant damage and yield response	Randolph, Peairs, Kroening, Armstrong, Hammon, Walker and Quick (2003)
Categories of resistance at different growth stages	Hawley, Peairs and Randolph (2003)
Possible roles esterase, glutathione S transferase and superoxide dismutase (detoxification enzymes)	Ni and Quisenberry (2003)
Differential colonisation by two biotypes	Qureshi *et al.* (2005)
Yield response and categories of resistance	Randolph *et al.* (2005)

Hydrogen peroxide has been shown to signal the induction of downstream defence reactions in TugelaDn, with salicylic acid acting as a later signal for systemic acquired resistance (Mohase and Van der Westhuizen, 2002). Additionally increased levels of salicylic acid inhibit catalase activity,

which in turn leads to elevated levels of hydrogen peroxide and consequent amplification of the resistance response. The rapid induction of reactive oxygen species (ROS) such as hydrogen peroxide is known as oxidative burst and has been described for plant resistance to both pathogens and herbivores. The balance between production and metabolism of ROS is important to prevent damage to cells. Moloi (2002) as cited by Van der Westhuizen, 2005 reported the induction of ROS scavenging enzymes with anti-oxidative action shortly after the induction of ROS generating enzymes, confirming the signalling role in *D. noxia* resistance. Lipoxygenase activity was also found to be selectively induced in infested TugelaDn wheat and is also thought to act as signal molecule in activating defence reactions through the 9-HPOD pathway (Van der Westhuizen, 2005). The observed biochemical responses as described above appear to form part of a combined defence mechanism closely resembling plant defence responses to pathogens.

Hydroxamic acids are present in cereals as ß-glucosides which are enzymatically converted to the corresponding aglycons when plant tissue is damaged (Virtanen and Hietala as cited by Mayoral, Tjalingii and Castañera (1996)). Main aglycones found in cereals are DIMBOA and DIBOA. These compounds are known to confer resistance to a wide range of natural enemies of plants including chewing and sap sucking insects as well as bacterial and fungal diseases (Gianoli and Niemeyer, 1998). A negative correlation was found between *D. noxia* population and DIBOA content of *Hordeum* seedlings. Nicol, Copaja, Wratten and Niemeyer (1992) screened worldwide wheat cultivars for hydroxamic acid levels finding that susceptible Betta had a DIMBOA level of 1.29 mmol.kg^{-1} fresh weight while that of susceptible Tugela was 2.00 mmol.kg^{-1} and that of the resistant donor accession SA 2199 [PI 262660] 2.15 mmol.kg^{-1} all of which fall in the moderate level as defined by Givovich and Niemeyer (1996).

The study of aphid-plant interactions using EPG

Prado (1997) defines aphid-plant interaction as comprising of host plant attraction, plant penetration, sap feeding by the aphid and the reactions to these activities by the plant. This is an extremely complex process that has been studied extensively for various aphid host-plant combinations but is not yet entirely understood (Caillaud and Niemeyer, 1996).

Study of the feeding behaviour of piercing-sucking insects like homopterans, is difficult because once the insect inserts its stylets into the plant tissue, relevant behaviours occur within the opaque food substrate and are not directly observable (Walker, 2000). Homopteran probing can however be effectively studied using the electrical penetration graph (EPG) technique (McLean and Kinsey, 1964; Tjallingii, 1978, 1985a, 1985b, 1988) and this method is increasingly being used to study aphid–plant interactions.

Initial attempts at electronic monitoring of insect probing were made in the early 1960's (McLean and Kinsey, 1964) and with time the systems have been further developed and refined. In principle

this technique works by connecting the aphid and the plant substrate into an electric circuit, which is completed when the aphid stylet penetrates the plant to feed. A thin gold wire (8-20 µm in diameter) is glued to the insect's dorsum using water based conductive silver glue and Tjallingii (1986) showed that this does not influence feeding behaviour significantly if correctly attached. A second electrode is connected to the plant or plant substrate. A small voltage (either AC or DC depending on the system used) is applied across the insect and the substrate. Completion of the circuit occurs when aphid stylets penetrate the plant, the current flows and a signal can be recorded. As the electrical impedance fluctuates in the insect-substrate circuit, these impedance fluctuations are superimposed on the rate of charge flow or current in the circuit. By converting the current fluctuations to voltage fluctuations and amplifying the voltage level the impedance changes become signals that can be observed and recorded using electronic devices. The DC system records two signal components originating from the insect-plant interaction namely the resistance or conductivity component (R) and the electromotive forces (emf) actively generated by the insect-plant combination, while the AC system records only the fluctuating voltage over time caused by changes in the electrical resistance of the insect-plant combination (R). Certain repetitive or periodic impedance changes have been correlated with specific behaviours (probing, salivation and ingestion) and with the penetration of certain plant tissue (Kimsey and McLean, 1987) and as systems have been improved and fine-tuned new waveforms and details could be correlated with previously unknown probing activities (Tjallingii, 2000). Some confusion can exist due to existence of both AC and DC systems each with their own peculiarity. The respective equivalents for the AC and DC waveforms are as follows: Salivation (S) for pathway phase (ABC); phloem ingestion (PI) or committed phloem ingestion (CPI) for phloem phase (E); non-phloem ingestion (NPI) for xylem phase.

The EPG technique has applications in the study of virus transmission (Woodford and Mann, 1992; Harrewijn, de Kogel and Piron, 1998), the influence of water deficit (Al-Dawood, Radcliffe, Backus and Koukkari, 1996), the effect of anti-feedant compounds and mineral oils (Powell, Hardie and Pickett, 1998), insecticides [Pymetrozine (Harrewijn and Kayser, 1997), Imidacloprid (Woodford and Mann, 1992; Epperlein and Jaschewski, 1997)] as well as the clarification of the insect-host plant interaction. EPG's provide the opportunity of localising the resistance mechanism in the plant, be it mechanical or chemical properties of plant tissues (phloem, cuticle, epidermis, mesophyll) (Van Helden and Tjallingii, 2000) thus facilitating the use of pest resistance in crops, an environmentally responsible strategy which is increasingly being deployed for the control of agriculturally significant pests (Van Helden and Tjallingii, 2000; Walker, 2000). EPG's have been used to study host plant resistance to many hemipterous pests amongst others spotted alfalfa aphid, *Therioaphis maculata,* on alfalfa (Nielson and Don, 1974); Melon aphid, *Aphis gossypii,* on muskmelon (Kennedy, McLean and Kinsey, 1978; Klinger, Powell, Thompson and Isaacs, 1998); brown planthopper, *Nilaparvata lugens,* on rice (Velusamy and Heinrichs, 1986); leafhopper, *Nephotettix virescens*, on rice (Rapusas and Heinrichs, 1990); black cowpea aphid, *Aphis cracivora,* on cowpea (Mesfin Thottapilly and Singh, 1992); Greenbug, *Schizaphis graminum,* on wheat (Morgham, Richardson, Campbell and Eikenberry, 1992); cabbage aphid, *Brevicoryne*

brassicae, on cabbage (Gabrys, Tjallingii and Van Beek, 1997); English grain aphid, *Sitobion avenae,* on wheat and triticale (Leszczyński, Urbańska, Rozbicka, and Matok, 1997); green peach aphid, *Myzus persicae,* on *Prunus* (Sauge, Kervella and Rahbé, 1998) and sweetpotato whitefly, *Bemisia tabaci,* on tomato (Lei, Tjallingii and Van Lenteren, 1998; Jiang, Lei, Collar, Martin, Muñiz and Fereres 1999).

Certain accepted "conventions" regarding EPG have arisen over time and are listed below: The non-probing period before the first probe is regarded as a an unreliable parameter as it appears highly sensitive to the aphid's pre-treatment (Tjallingii and Mayoral, 1992). Probes equal to or less than 2 min (Van Hoof, 1958 as cited by Gabrys *et al*., 1997) do not go beyond one cell layer. The duration of waveform E2 for 10 min or more is referred to as sustained phloem ingestion and considered to reflect sieve element acceptance (Tjallingii and Mayoral, 1992).

Russian wheat aphid probing behaviour

Electronic monitoring of *D. noxia* feeding has been reported on wheat, oats, rye (hosts) and sorghum (nonhost) by Girma, Wilde and Reese (1992), on susceptible wheat as well as resistant and susceptible slender wheatgrass accessions by Kindler, Greer and Springer (1992), on barley by Webster, Porter, Baker and Mornhinweg (1993), on insecticide treated wheat by Burd, Elliott and Reed (1996), on resistant wheats and triticales by Givovich and Niemeyer (1996), on bread wheat, hard wheat, triticale, rye and barley with different hydroxamic acid levels by Mayoral *et al.* (1996), on *D. noxia* resistance donor accessions by Ni and Quisenberry (1997b) and on water stressed, *D. noxia* resistant barley by Brewer and Webster (2001).

Most EPG studies conducted previously show that resistance to *D. noxia* manifests in an effect on the phloem feeding of the aphids. Girma *et al*. (1992) reported that D. noxia salivated more and ingested less when feeding on a nonhost and that it took four times longer to locate the phloem and achieve committed phloem ingestion while there were no differences in phloem ingestion of *D. noxia* on wheat, rye and oat host plants. Kindler *et al*. (1992) reported significantly longer phloem feeding on the most susceptible wheatgrass accession and an increase in nonfeeding behaviour on the resistant wheatgrasses. A higher frequency of baseline activity, salivation and nonphloem ingestion on resistant barley lines was reported Webster *et al*. (1993) with *D. noxia* spending significantly more time ingesting from the phloem of susceptible lines. *D. noxia* on resistant lines took longer to reach first committed phloem ingestion on resistant barley lines. Burd *et al*. (1996) reported that *D. noxia* on untreated susceptible wheat spent ≈ 35% of the total duration of EPG study in low amplitude salivation activities, with no significant differences in feeding behaviour being observed between Gaucho-treated and untreated wheat. In two studies higher levels of hydroxamic acid in test entries led to a delay in attaining sustained phloem feeding (Givovich and Niemeyer, 1996), less probing and a lower percentage of aphids reaching sustained phloem ingestion (Mayoral *et al*., 1996). Ni and Quisenberry (1997b) reported that *D. noxia* probed

significantly more diurnally than nocturnally, and that diurnal probes were significantly shorter than nocturnal ones. No significant differences were reported between the resistance donors PI 137739 and PI 262660, however total probing duration, duration per probe and duration of salivation and ingestion per probe was significantly lower on PI 137739 than on the susceptible control Arapahoe wheat while PI 262660 did not differ from Arapahoe. It was also reported that *D. noxia* seemed to feed more nocturnally on PI 137739 and Halt to compensate for less diurnal feeding. Brewer and Webster (2001) reported that *D. noxia* took longer to first enter the sieve element phase of feeding and that the duration of the sieve element phase on resistant 'STARS-9301B' barley was shorter than on susceptible 'Morex'.

Aims, scope and contents of this study

In an attempt to better understand host plant resistance to *D. noxia ex* donor accessions PI 137739 (*Dn1*) and PI 262660 (*Dn2*) and their use as control measures in the form of resistant cultivars, this study aims to investigate the genetic variability of *D. noxia* resistant and susceptible near-isogenic lines. The characterisation of the mechanisms of resistance to *D. noxia*, the influence of resistance on population development of *D. noxia* in the field and the influence of resistance on the probing behaviour of *D. noxia* in near-isolines will be investigated. This may assist breeders in future efforts to better understand and therefore successfully exploit genetic resistance to this damaging pest. In addition, quantifying the yield loss due to *D. noxia* damage, in commercially available resistant cultivars will illustrate the practical application of this resistance under field conditions when deployed in varying genetic backgrounds.

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Chapter 2

Mechanisms of resistance and their influence on the population development of Russian wheat aphid on susceptible and resistant wheat lines under field conditions.

Abstract

Diuraphis noxia is the most serious pest of wheat in South Africa. The cultivars Betta and Tugela were used as susceptible controls while two BC_3 lines of Tugela with resistance *ex* the *D. noxia* resistance sources SA 1684 [PI 137739 (Dn1)] and SA 2199 [PI 262660 (Dn2)] were studied. The mechanism of resistance was studied under controlled conditions to determine antibiosis, antixenosis and tolerance of the advanced germplasm lines. A randomised block design trial with six replications was carried out for two consecutive seasons, 1993 and 1994, to determine the influence of these resistant advanced lines on the population development of *D. noxia* under field conditions before the use of resistant cultivars became a widespread control option for this pest. Mechanism of resistance studies conducted under controlled conditions showed antibiosis present in both resistant lines. This is consistent with the data obtained when the original sources of resistance were tested and also correlates well with the lower *D. noxia* numbers that were recorded on the resistant lines under field conditions. Significantly more *D. noxia* were recovered from 2199/Tugela in the antixenosis experiment than from any of the other test entries indicating a lack of antixenosis in this line. Although not significantly so, the least *D. noxia* were recovered from 1684/Tugela in the antixenosis experiment, possibly an indication of weak antixenosis in this line, which may in part account for the lower populations of *D. noxia* recorded under field conditions. Although neither of the resistant lines showed high levels of tolerance to *D. noxia* infestation, 1684/Tugela was able to retain the same leaf area and plant dry mass as 2199/Tugela under a much higher aphid infestation level and both lines were significantly more tolerant than the susceptible control Betta, but did not differ from the other susceptible control, Tugela. In the field trial, the 1993 season was characterized by high yields and very low *D. noxia* numbers in contrast to the 1994 season where severe *D. noxia* infestations and lower yields occurred. The percentage of infested tillers was higher on the susceptible cultivars Betta and Tugela than on the resistant lines during both seasons. During both seasons, the percentage of tillers infested on the resistant lines was much lower than on the susceptibles, reaching maximums of 12% and 50% during 1993 and 1994 respectively. The number of *D. noxia* per tiller was also lower on the resistant lines during both seasons varying from a mean maximum of three aphids per infested tiller on the resistant lines to a mean maximum of 50 aphids per infested tiller on the susceptible lines. The data collected shows that *D. noxia* resistance lowers both the percentage of tillers infested and number of *D. noxia* per infested tiller, resulting in higher yields from resistant lines.

Key words: antibiosis, antixenosis, *Diuraphis noxia*, host plant resistance, PI 137739 (*Dn1*), PI 262660 (*Dn2*), tolerance, wheat

Introduction

The most serious pest of wheat produced in the summer rainfall region of South Africa is the Russian wheat aphid, *Diuraphis noxia (Kurdjumov),* which was first reported as a pest of wheat in the Free State Province in 1978 (Walters, 1984). This pest has been the target of a highly successful integrated control programme (Marasas, 1999), utilising resistant cultivars, natural enemies, cultural practices and insecticides, which has developed progressively over the past 13 years since the release of the first resistant cultivar, Tugela-DN, in 1992 by ARC-Small Grain Institute (Du Toit, 1993; Van Niekerk, 2001).

In South Africa resistant cultivars have been seen as an ideal management option for *D. noxia* because they are one of the most desirable alternatives to insecticides offering both economically justifiable and effective control of this pest. The identification of genetic resistance to *D. noxia* in South Africa (Du Toit, 1987; 1988; 1992), led to the introduction of resistance into wheat lines with more acceptable agronomic backgrounds, through backcrossing. This facilitated the development of resistant cultivars by breeders and by 2005 a total of 26 cultivars with *D. noxia* resistance had been released in South Africa namely Betta-Dn, Caledon, Elands, Gariep, Komati, Limpopo, Matlabas, Nossob, PAN3235, PAN3364, SST124, SST322, SST 333, SST334, SST347, SST363, SST367, SST399, SST935, SST936, SST966, SST972, SST983, Tarka and Tugela-Dn (Dr A. Barnard, personal communication[2]). Marasas Anandajayasekeram, Tolmay, Martella, Purchase and Prinsloo (1997) confirmed *D. noxia* resistant cultivars to have a yield advantage over susceptible cultivars in farmers' fields and established that the adoption-rate of *D. noxia* resistant cultivars in South Africa was limited only by the availability of seed. Due to the rapid adoption of resistant cultivars, the average area treated with insecticides decreased from 85% in 1990 to 30% in 1997 (Marasas, 1999) with a further decrease of 16% projected to take place by 2000. In addition to this the number of sprays per year decreased from four during the 1990-1992 seasons to only one in seasons subsequent to 1996. It is now estimated that approximately 70% of the area planted to wheat annually is planted to resistant cultivars.

Host plant resistance has been used as a control measure for various agricultural pests for many years (Smith, 1989). Painter (1951) explained host plant resistance by using three functional categories, namely antibiosis, non-preference (antixenosis) and tolerance. Antibiosis describes the negative influence of the plant on the biology of an insect attempting to use that plant as host (Smith, 1989). This may be expressed as reduced body size and mass, prolonged periods of development in the immature stages, reduced fecundity or failure to pupate or eclose. Antixenosis, the inability of a plant to serve as a host, is caused by physical or chemical plant factors that repel or deter insects from feeding or oviposition (Smith, 1989). Tolerance indicates the plant's ability to withstand or compensate for insect damage (Smith, 1989). Known components of this form of resistance include general vigour, compensatory growth, wound

healing, mechanical support in tissues and organs and changes in photosynthetic partitioning. Environmental factors, however may affect tolerance more than other types of resistance (Pedigo, 1989). The mechanism of resistance in a specific line will influence the efficacy of the line in controlling field populations of the pest and may in part determine the longevity of the resistance under field conditions by influencing the formation of a biotype (Gallun, 1972). Many factors play a role in the expression of host plant resistance and its effect on the target pest when deployed in resistant cultivars in the field.

Du Toit (1989a) reported the resistance in PI 137739 and PI 260660 to *D. noxia* to be governed by different, single dominant genes. These genes were designated Dn1 and Dn2 respectively and the mechanisms of resistance in the donor lines shown to be antibiosis and antixenosis (Du Toit, 1987, 1989b). Various other authors also studied these original sources of *D. noxia* resistance, PI 137739 and PI 262660. Smith, Schotzko, Zemetra and Souza (1992) evaluated these lines under greenhouse conditions in Idaho to determine the categories of resistance. Based on percentage reduction in plant height, it was concluded that both lines possessed a significant level of tolerance to *D. noxia* feeding. *D. noxia* maintained on these lines displayed reduced reproductive rates twenty-one days after infestation, indicating the presence of low-level antibiosis. *D. noxia* on PI 137739 were found to have a significantly lower reproduction rate than on PI 262660 and Stephens, the susceptible control, in a trial conducted by Quisenberry and Schotzko (1994). This indicated that PI 137739 showed antibiosis in contrast to PI 262660 which had higher plant growth, dry weight and moisture while expressing higher leaf chlorosis and mid-leaf rolling indicating tolerance. Mowry (1994) found that antibiosis in the lines PI 137739 and PI 262660 could not be detected statistically when uninfested plants were compared to susceptible controls, but that *D. noxia* performance was significantly less on these lines when Barley Yellow Dwarf Virus (BYDV) - infested plants were compared, indicating that plant stress influences the expression of *D. noxia* antibiosis.

Despite various studies on the original accessions PI 137739 and PI 262660, the influence of agronomically adapted, resistant wheat lines containing these genes on field populations of *D. noxia* is not well documented. Therefore, in addition to describing the mechanisms of resistance in advanced breeding lines containing the resistance *ex* PI 137739 and PI 262660, this study aims to show the effect of this germplasm on the population development of *D. noxia* under field conditions in South Africa prior to and a decade after, the widespread use of resistant cultivars for the control of this pest.

Material and methods

Two resistant, advanced breeding lines namely SA1684/4*Tugela and SA 2199/4*Tugela containing the resistance *ex* SA 1684 [PI 137739 (Dn1)] and SA 2199 [PI 262660 (Dn2)] (Du Toit,

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1987, 1989a, 1989b) respectively, were used throughout the study. These lines were developed as part of an extensive backcross-breeding programme aimed at incorporating *D. noxia* resistance in well adapted germplasm (Du Toit, 1993). Homozygous lines, produced by selfing BC_3F_1 plants twice, were tested for resistance to *D. noxia* and agronomic traits under field conditions in the Free State Province whereafter they were included in advanced trials for several years prior to being used in this study. These lines will be referred to as 1684/Tugela and 2199/Tugela in this manuscript. Two susceptible cultivars Betta and Tugela were used as control throughout allowing for comparison with data collected by Aalbersberg (1987).

Mechanisms of resistance

A six week screening procedure described by Tolmay, Van der Westhuisen and Van Deventer (1999) using the colony count technique for antibiosis, a completely random free choice experiment for antixenosis and a three week tolerance test measuring initial and final plant height, initial and final *D. noxia* infestation, damage rating, leaf area and dry plant mass was used as the basis methodology for these studies. Modifications, where applicable are noted in the text.

Antibiosis

Four 1 litre pots (replications) per entry, each containing one plant, were used in a randomised block design in the greenhouse, which was maintained at 22°C/15°C day/night with natural photoperiod. Adequate fertilizer was mixed with the soil prior to planting. Four pre-conditioned (aphids reared on applicable test entry prior to test), fourth instar *D. noxia*, were placed on each plant at the two-leaf stage. Prior to infestation the total mass of the four *D. noxia* was determined using an Sartorius five decimal analytical balance. Each plant was covered with a cream coloured PVC pipe cage with large ventilation windows, covered with gauze. Fourteen days after infestation, the cages were opened, the plants cut off and the final number and final mass of *D. noxia* (all instars) determined. The experiment was repeated three times and data for the variables initial *D. noxia* mass (mg), total final *D. noxia* mass (mg), final number of *D. noxia* and mean *D. noxia* mass (mg) were analyzed in randomised block with 12 replicates using "ANOVA" (GenStat, 2000). LSD_T ($p < 0.05$) was used to compare means.

Antixenosis

Ten plants of each of the entries were planted in a completely randomised design within plastic seed trays 300 mm x 270 mm x 100 mm. At the two-leaf stage (10 days after planting) the seed trays were transferred from the greenhouse maintained at a temperature of 22°C (day) and 15°C (night) with a natural photoperiod, to a growth chamber maintained at a constant temperature of 18°C with a photoperiod of 12:12 L:D. *D. noxia* for the experiments were pre-conditioned on each of the four test entries prior to performing the experiment. Four seed trays per type of pre-conditioned *D. noxia* were infested shortly after transfer to the growth chamber. Two hundred apterae *D. noxia* were distributed evenly over each of the seed trays giving a mean infestation of five *D. noxia* per plant. Twenty-four hours after infestation, counts were made. Newborn nymphs (*circa* <24h old) were deemed not to have made a choice regarding the host plant they occured on,

and were therefore not counted. Forty replications (plants) per test entry, per type of pre-conditioned *D. noxia* were used for this experiment. The Chi-squared test using FREQ.EXE procedure (Van Ark, 1992) was used to compare the observed number of *D. noxia* per test entry, per pre-conditioning treatment, using a 4 x 4 contingency table. Chi-squared was tested at $p < 0.05$ and df=9.

Tolerance

A randomised block design with 10 replications was used where an uninfested control plant was included for each infested plant of the four test entries. Data from the infested plants were expressed as a percentage of the uninfested control plant with the exception of aphid counts. Two seeds of each of the test entries were planted in sand, in 2 litre pots in the greenhouse and covered with plastic pipe cages (79 cm tall and 11.5 cm in diameter) made from cream coloured PVC pipe with ventilation openings covered in organza. All plants received automatic irrigation and fertilization (Chemicult). After plants had emerged, the strongest plant per pot was chosen and the other removed. Prior to infestation the plant height, was determined for all plants. Ten plants of each of the test entries were infested with a single fourth or fifth instar *D. noxia* per 2 cm plant height. The cages were replaced and left in position until it was noted that the susceptible plants were showing typical symptoms of *D. noxia* damage (three weeks after infestation). Data collection was started simultaneously for all entries and the following determined: Plant height (cm), number of *D. noxia* per plant (all instars), leaf area using a Licor leaf area meter (cm^2) and the plant dry mass (g). The "NORMTEST" procedure (GenStat, 2000) was performed on all data to determine distribution of data points. Data was analysed by performing "ANOVA" (GenStat, 2000), and LSD_T ($p < 0.05$) was then used to identify significant differences.

<u>*D. noxia* population development under field conditions: 1993 - 1994 seasons</u>

A randomised block design trial with six replicates was carried out for two consecutive seasons, 1993 and 1994 at the ARC-Small Grain Institute near Bethlehem [28°10'S, 28°18'E]. Plots measured 10 m by 4.5 m to allow for destructive sampling. Each week from the middle of September until the end of November, six plants were randomly sampled from each plot. The number of tillers per plant and the number of infested tillers were determined which was then used to calculate the percentage infested tillers. The number of *D. noxia* per infested tiller was also counted. The total infestation for the season was calculated by summing the weekly infestation. Data were analysed using 'ANOVA' and means were compared using the Bonferroni (Dunn) T test, with $p \leq 0.05$ and df=15. The yield was determined and analysed with 'ANOVA' using Tuckey's Least Significant Difference (LSD_T) with $p \leq 0.05$ and df=23 to compare means (GenStat, 2000).

<u>*D. noxia* population development under field conditions: 2004 season</u>

The field experiment conducted in 1993/1994 was repeated in 2004 to determine whether the reaction of *D. noxia* to the advanced breeding lines was still the same as it was prior to the

widespread deployment of resistant cultivars. A randomised block design trial with four replicates, not six as previously, was planted at the ARC-Small Grain Institute near Bethlehem [28°10'S, 28°18'E]. Plots were 5m by 2.25m and all *D. noxia* counts were performed *in situ*. Each week from the middle of September until the end of October, ten plants were randomly sampled in each plot. Extremely low levels of *D. noxia* infestation were monitored in September and October before the trial was terminated due to insufficient data and drought damage.

Results and Discussion

Mechanisms of resistance

Antibiosis:

Data are shown in Table 2.1. There was no significant difference in the initial mass of the *D. noxia* used to infest the test entries. After 14 days the total final mass of *D. noxia* recovered from Betta was significantly higher than that recovered from Tugela, 1684/Tugela and 2199/Tugela, which did not differ significantly from each other. The final number of *D. noxia* recovered did not differ significantly between entries. However, the mean final mass of *D. noxia* (calculated as the total final mass/final number) showed significant differences with the heaviest *D. noxia* being recovered from Betta, followed by those recovered from Tugela and 1684/Tugela which were intermediate and did not differ from each other, with *D. noxia* recovered from 2199/Tugela having significantly lower mean final mass than all other entries. This would indicate antibiosis most likely due to a lengthening of the duration of instars (slower development) or reduction in size (smaller body mass) as the number of *D. noxia* recovered from 2199/Tugela was not different to the number recovered from other lines. Although not significantly different from the susceptible control Tugela, the resistant line 1684/Tugela gave a significantly lower total and mean final mass of *D. noxia* than the susceptible control Betta, indicating the presence of low levels of antibiosis in this line.

TABLE 2.1: The initial mass of *D. noxia*, final number, total and mean final mass of *D. noxia* from colony count experiment. (^{abc} means within columns, without letters in common, differ significantly LSD_T ($p < 0.05$))

Test entries	Initial *D. noxia* mass (mg)	Final number *D. noxia*	Total final *D. noxia* mass (mg)	Mean final *D. noxia* mass (mg)
Betta	0.895a	91a	13.52a	0.1536a
Tugela	0.884a	81a	10.75b	0.1306b
1684/Tugela	0.916a	82a	9.83b	0.1238b
2199/Tugela	0.914a	88a	8.81b	0.9909c
df	33	32	32	32
% CV	6.0	23.2	26.8	19.8
LSD_T	0.0452	16	2.39	0.2092

Antixenosis:

Antixenosis was determined using the completely random free choice experiment. The number of *D. noxia* recovered per cultivar/line for each pre-conditioning treatment, 24 hours after infestation is shown in Table 2.2a. The frequencies are not significantly different, (Chi-squared = 9.846 and Tabled Chi squared = 16.919, $p<0.05$, df=9) showing that no preference or non-preference for any of the entries exists based on the preconditioning of the *D. noxia* used in the experiment. Table 2.2b shows the number of *D. noxia* recovered of the total released onto the flats. Of the 12800 *D. noxia* released onto the flats only 19.2% were recovered from the test entries. Chi-squared test showed significant differences for the following comparison of pairs of rows at a test level of $p=0.05$ and a tabled Chi-squared value of 6.982; Betta vs 2199/Tugela (15.118), Tugela vs 2199/Tugela (9.593) and 1684/Tugela vs 2199/Tugela (26.627). Significantly more *D.noxia* were recovered from 2199/Tugela than from any of the other entries indicating that this line is more attractive to colonisation by *D. noxia* than the other resistant line or either of the susceptible cultivars.

TABLE 2.2a: The number of *D. noxia* recovered per cultivar/line for each pre-conditioning treatment, 24 hours after infestation.

Test entries	Pre-conditioned *D. noxia*				
	Pre-Betta	Pre-Tugela	Pre-1684/Tugela	Pre-2199/Tugela	Total
Betta	170	159	132	124	585
Tugela	169	170	126	145	610
1684/Tugela	151	155	102	138	546
2199/Tugela	171	196	166	178	711
Total	661	680	526	585	2452

TABLE 2.2b: The number of *D. noxia* recovered per cultivar/line, of the total released onto the flats.

Test entries	Number of *D. noxia* recovered from test entries	Number of *D. noxia* unaccounted for	Number *D. noxia* released onto flats
Betta	585	2615	3200
Tugela	610	2590	3200
1684/Tugela	546	2654	3200
2199/Tugela	711	2489	3200
Total	2452	10348	12800

Tolerance:

Plant height was determined prior to the onset of the trial and plants to be infested were compared to those to remain uninfested. No significant differences occurred although Betta and Tugela were taller than 1684/Tugela and 2199/Tugela. Data is shown in Table 2.3. The susceptible controls Betta and Tugela as well as the resistant line 1684/Tugela showed significantly higher *D. noxia* infestation levels at the end of the trial than the resistant line 2199/Tu (Table 2.4), confirming antibiosis as mechanism of resistance in this line. The final leaf area and plant dry mass are given in Table 2.5. Betta was most damaged by *D. noxia* with significantly the smallest leaf area and the least plant dry mass at the end of the trial. There was no difference between the resistant Tugela lines, indicating that 1684/Tugela is more tolerant than 2199/Tugela as a significantly higher infestation caused the same level of damage.

TABLE 2.3: The plant height of test entries at the onset and end of the tolerance trial.

Test entries	Initial Plant height (cm)[1]		Final Plant height (cm)[2]		Final plant height (% of uninfested control)[3]
	Uninfested	To be infested	Uninfested	Infested	
Betta	9.05a	9.00a	27.10a	15.30b	58.6a
Tugela	9.15a	8.90a	28.50a	17.05b	60.9a
1684/Tugela	8.50a	8.00a	28.00a	15.80b	57.3a
2199/Tugela	8.15a	7.85a	28.05a	20.65b	75.1a
df	63		63		27
% CV	14.5		19.1		31.6
LSD_T	1.11		3.85		18.2

[1 & 2] (^{abc} means within rows for each parameter, without letters in common, differ significantly LSD_T ($p < 0.05$))

[3] (^{abc} means within columns, without letters in common, differ significantly LSD_T ($p < 0.05$))

TABLE 2.4: The initial and final infestation rates of the tolerance trial. (^{abc} means within columns, without letters in common, differ significantly LSD_T ($p < 0.05$))

Test entries	Initial infestation	Final infestation
	Number of *D. noxia* /plant	Number of *D. noxia* /plant
Betta	4.1a	145.1a
Tugela	4.2a	99.1a
1684/Tugela	3.8a	118.1a
2199/Tugela	3.6a	29.7b
df	27	27
% CV	18.0	52.4
LSD_T	0.6	47.1

TABLE 2.5: Leaf area and plant dry mass of resistant lines and susceptible control in a tolerance test. (^{abc} means within columns, without letters in common, differ significantly LSD_T ($p < 0.05$))

Test entries	Leaf area (% of uninfested control)	Plant dry mass (% of uninfested control)
Betta	18.7a	15.3a
Tugela	46.5b	59.8b
1684/Tugela	45.7b	51.3b
2199/Tugela	51.0b	47.4b
df	27	27
% CV	41.5	63.8
LSD_T	15.4	25.4

D. noxia population development under field conditions: 1993 - 1994 seasons

The 1993 season was characterized by high yields and low *D. noxia* numbers (Table 2.6) in contrast to the 1994 season where severe *D. noxia* infestations and lower yields occurred (Table 2.7). During both seasons the total percentage of infested tillers was significantly higher on Betta and Tugela. The number of *D. noxia* per infested tiller was also significantly higher on the susceptible controls during both seasons. The hectolitre mass of Tugela was significantly lower than all other lines in both seasons. Betta and 1684/Tugela had the highest hectolitre mass in 1993, and 1684/Tugela and 2199/Tugela had the highest hectolitre mass in 1994.

TABLE 2.6: Yield ($t.ha^{-1}$), hectolitre mass ($kg.hl^{-1}$) and infestation expressed as total % infested tillers and number of *D. noxia* per infested tiller for the 1993 season. (^{abc} means within columns, without letters in common, differ significantly LSD_T ($p < 0.05$))

Test entries	Yield ($t.ha^{-1}$)	Hectolitre mass ($kg.hl^{-1}$)	Infestation	
			Total % Infested tillers for season	Number of *D. noxia* per infested tiller
Betta	3.352c	76.56c	202.87a	7.94a (63.45)
Tugela	4.013b	72.55a	176.38a	7.86a (61.77)
1684/Tugela	5.116a	76.63c	46.88b	4.86b (23.58)
2199/Tugela	4.289b	73.85b	22.58b	3.88b (14.43)
df	15	15	15	15
% CV	8.4	1.0	39.7	44.74
LSD_T	5.860	1.20	36.08	1.913

TABLE 2.7: Yield (t.ha^{-1}), hectolitre mass (kg.hl^{-1}) and infestation expressed as total % infested tillers and number of *D. noxia* per infested tiller for the 1994 season. (^{abc} means within columns, without letters in common, differ significantly LSD_T ($p < 0.05$))

Test entries	Yield (t.ha^{-1})[1]	Hectolitre mass[2] (kg.hl^{-1})	Infestation[3]	
			Total % Infested tillers for season	Number of *D. noxia* per infested tiller
Betta	0.612a	74.30b	443.57a	11.94a (142.6)
Tugela	0.625a	72.40a	455.10a	10.11a (102.2)
1684/Tugela	1.284b	77.00c	215.30b	3.26b (10.6)
2199/Tugela	1.313b	77.37c	123.99b	2.38b (5.6)
df	15	15	15	15
% CV	31.8	1.4	16.9	17.76
LSD_T	0.375	1.28	91.45	2.16

D. noxia numbers were lower during 1993 than during 1994 with the maximum percentage of infested tillers on the susceptible controls reaching 34% and 90% respectively. The percentage infested tillers is shown in Figures 2.1 (1993) and 2.2 (1994). The maximum percentage of infested tillers on the resistant lines was lower than on the susceptible controls reaching 12% and 50% during 1993 and 1994 respectively.

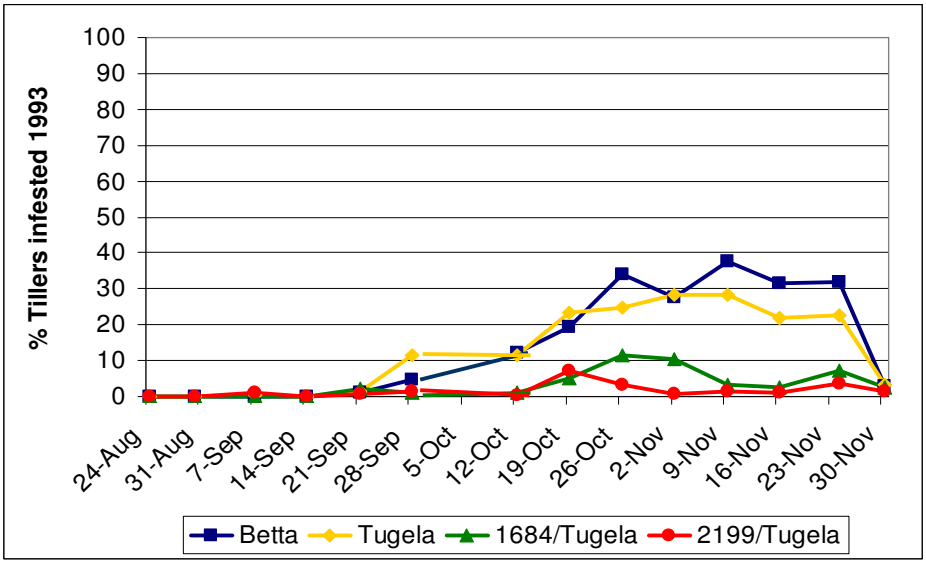

FIGURE 2.1: Percentage tillers infested with *D. noxia* in the 1993 season

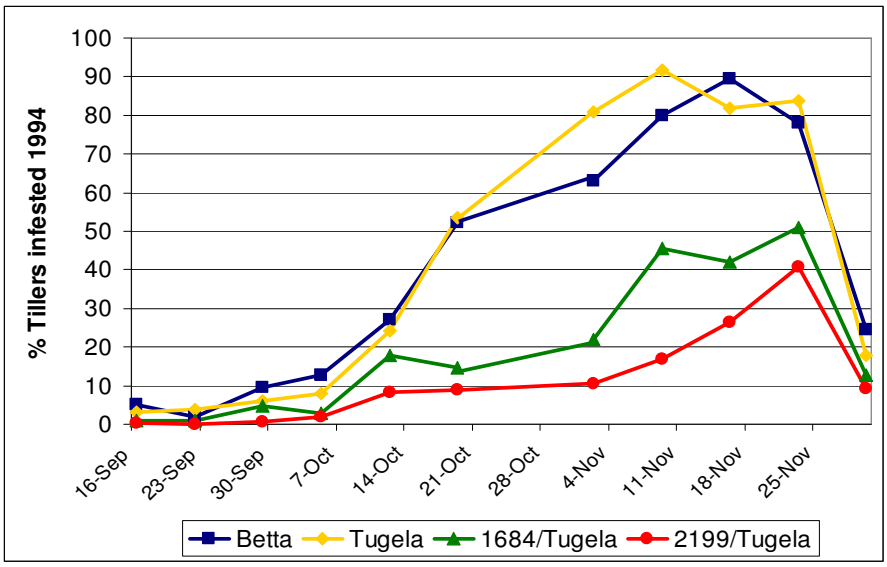

FIGURE 2.2: Percentage tillers infested with *D. noxia* in the 1994 season

The number of *D. noxia* per infested tiller was also much lower on the resistant lines during both seasons. The number of *D. noxia* per infested tiller is shown in Figures 2.3 (1993) and 2.4 (1994) respectively. *D. noxia* resistance lowers both the percentage of tillers infested and the number of *D. noxia* per infested tiller resulting in higher yields from resistant lines. The effect of resistance is more pronounced when high *D. noxia* infestations occur.

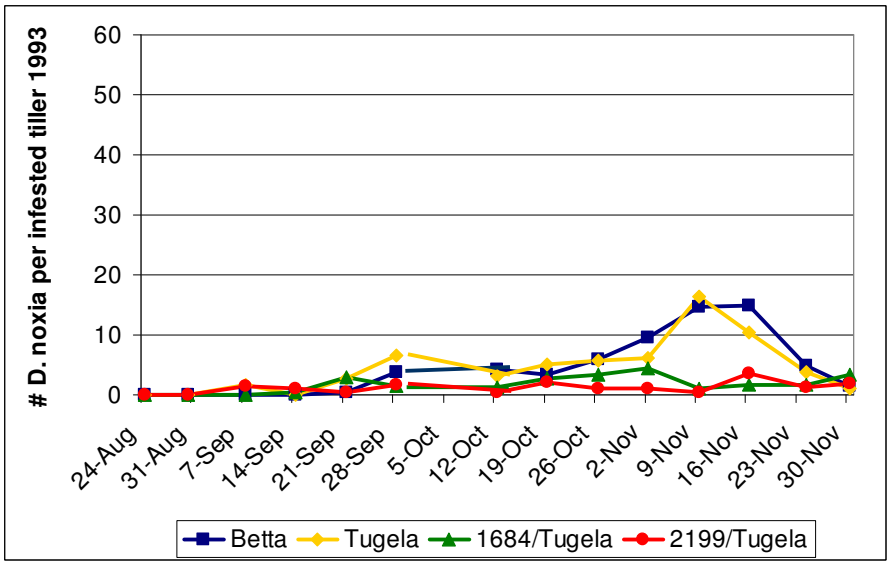

FIGURE 2.3: Number of *D. noxia* per infested tiller in the 1993 season

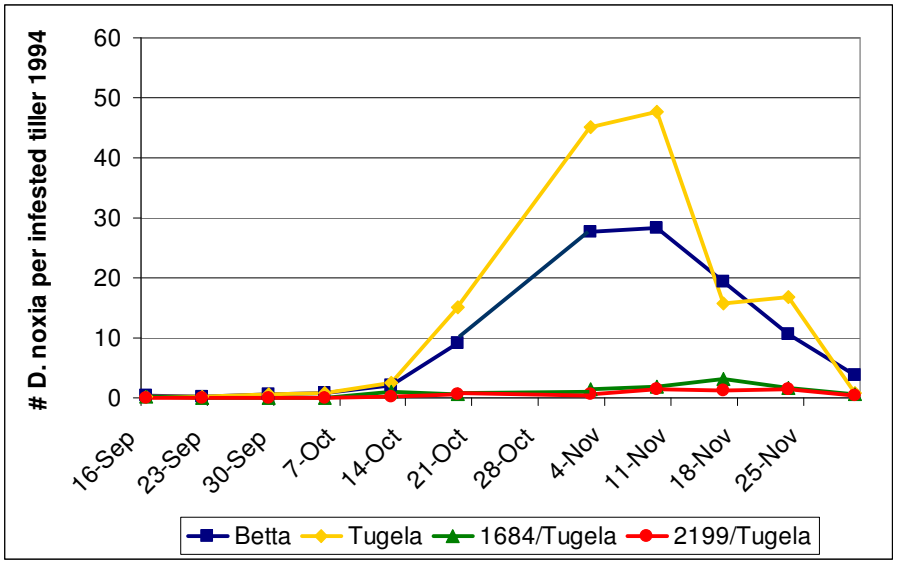

FIGURE 2.4: Number of *D. noxia* per infested tiller in the 1994 season

Yields were much higher during 1993 (Figure 2.5) than during 1994 (Figure 2.6). The difference in yield between the resistant lines and susceptible controls was more pronounced during 1994 when higher *D. noxia* infestation was present.

Aalbersberg (1987) studied the population build-up of *D. noxia* in the susceptible cultivar Betta from 1983-1985. It was found that the initial rapid increase of *D. noxia* on Betta began in mid-September and that peak aphid numbers were reached towards the end of October. A maximum number of 150 - 160 *D. noxia* per tiller were recorded on Betta in 1983/1984 and 100 % tillers were infested.

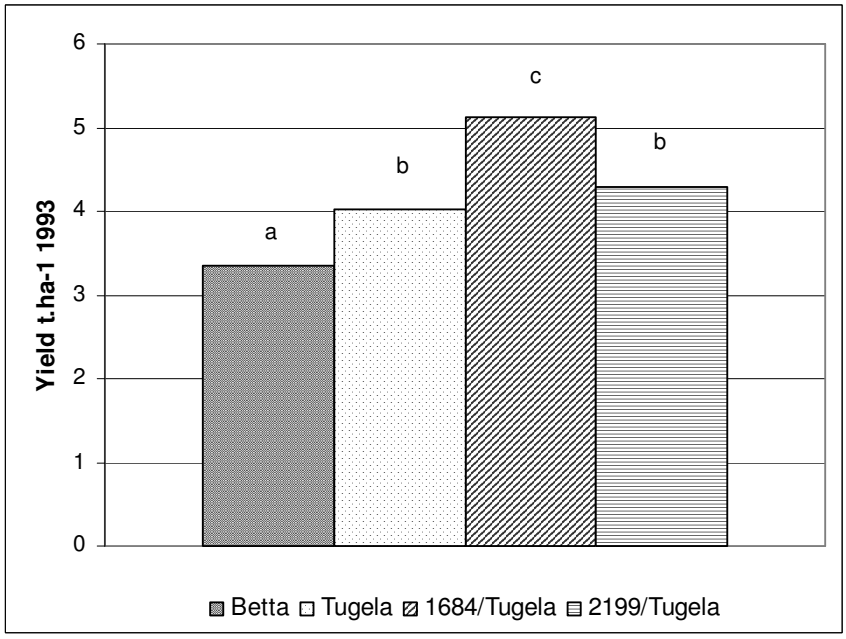

FIGURE 2.5: Grain yield ($t.ha^{-1}$) at Bethlehem, South Africa in the 1993 season. ^{abc} means without letters in common, differ significantly LSD_T ($p < 0.05$))

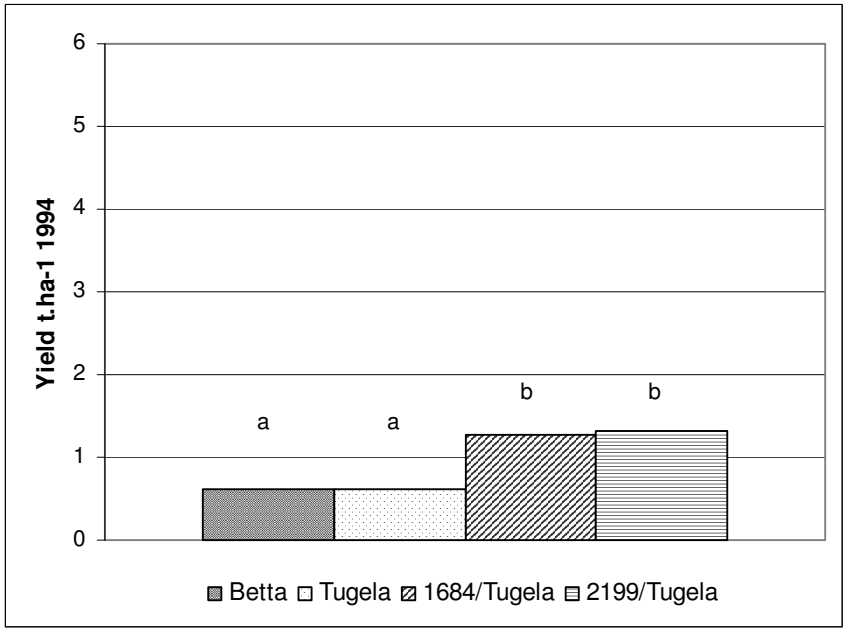

FIGURE 2.6: Grain yield ($t.ha^{-1}$) at Bethlehem, South Africa in the 1994 season. ^{abc} means without letters in common, differ significantly LSD_T ($p < 0.05$))

Conclusions

Mechanism of resistance studies conducted under controlled conditions showed antibiosis present in both resistant lines. This is consistent with the data obtained when the original sources of resistance were tested and also correlates well with the lower *D. noxia* numbers that were recorded on the resistant lines under field conditions. Although not significantly so, the line 2199/Tugela was less infested than the resistant line 1684/Tugela in both seasons in the field, a trend also found in the antibiosis tests indicating that the level of antibiosis may be slightly higher in 2199/Tugela. Significantly more *D. noxia* were recovered from 2199/Tugela in the antixenosis experiment than from any of the other test entries indicating a lack of antixenosis in this line. Nicol, Copaja, Wratten and Niemeyer (1992) screened worldwide wheat cultivars for hydroxamic acid levels finding that Betta had a DIMBOA (the main hydroxamic acid in wheat) level of 1.29 $mmol.kg^{-1}$ fresh weight while that of Tugela was 2.00 $mmol.kg^{-1}$ and that of the resistant donor line SA 2199 was 2.15 $mmol.kg^{-1}$. These concentrations are considered medium where a level of above 3.4 would be considered high (Givovich and Niemeyer 1996). DIMBOA has been found to exert both toxic and antifeedant effects on other grain aphids (Nicol *et al*., 1992) while Givovich and Niemeyer (1996) reported that higher DIMBOA levels in wheat seedlings led to lower mean relative growth rates of *D. noxia*.

Although not significantly so, the least *D. noxia* were recovered from 1684/Tugela in the antixenosis experiment, possibly an indication of weak antixenosis in this line, which may in part account for the lower populations of *D. noxia* recorded under field conditions. Although neither of the resistant lines showed high levels of tolerance to *D. noxia* infestation, 1684/Tugela was able to retain the same leaf area and plant dry mass as 2199/Tugela under a much higher aphid infestation level and both lines were significantly more tolerant than the susceptible control Betta, but did not differ from the other susceptible control, Tugela. Tolmay and Van Deventer (2005) reported that *D. noxia* infested Tugela-Dn, a sister line of 1684/Tugela, yielded 93.5%, 100% and 67.9% of the yield of its aphid free control in 2000, 2001 and 2003 respectively implying a compensation for or tolerance to *D. noxia* damage.

The 1993 and 1994 seasons in which population build-up of *D. noxia* was studied under field conditions were very different. High yields and very low *D. noxia* numbers characterized the 1993 season, in contrast to the 1994 season where severe *D. noxia* infestations and lower yields occurred due to very dry condition that prevailed in comparison to the wetter 1993. The percentage of infested tillers was higher on the susceptible cultivars Betta and Tugela than on the resistant lines during both seasons. During both seasons the percentage of tillers infested on the resistant lines was much lower, reaching maximums of 12% and 50% during 1993 and 1994 respectively. The number of *D. noxia* per tiller was also lower on the resistant lines during both seasons with a maximum average of 4.5 and 3.2 aphids per infested tiller on the resistant lines in 1993 and 1994 respectively. The data collected shows that *D. noxia* resistance lowers both the percentage of tillers infested and number of *D. noxia* per infested tiller, resulting in higher yields

from resistant lines. As found by Aalbersberg (1987), the initial rapid increase of *D. noxia* on all the test entries began in mid-September, but peak aphid numbers were only reached in early to mid-November in 1993 and 1994 in contrast to Aalbersberg (1987) who reported that peak numbers were reached towards the end of October in 1983 and 1984.

Data collected in 2004 reflects that extremely low population levels of *D. noxia* were present despite the dry conditions that prevailed and usually favour *D. noxia,* as was the case in 1994. This may be explained by the widespread use of resistant cultivars for *D. noxia* control, which has been estimated at approximately 70 % of the area planted to wheat in the Free State (Tolmay, 2001). This significantly hampers population build-up both during and between seasons, as most volunteer wheat, a significant between season host of the aphid (Kriel, Hewitt, De Jager, Walters, Fouchè and Van der Westhuizen, 1984), is resistant.

Usually more than one mechanism is present in a given line and the resistance reaction depends to some extent, on the genetic background of the line in which it is (Smith, 1989). Thus low to moderate levels (non-significant) levels of antibiosis, antixenosis or tolerance measured in controlled environment studies of advanced lines can manifest as biological and significant levels of field resistance, giving acceptable control of the target pest.

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Chapter 3

Initial exploratory study of electronic monitoring of Russian wheat aphid feeding behaviour on susceptible and resistant wheat lines.

Abstract

This chapter describes the electrical penetration graph technique using an initial exploratory study of *D. noxia* probing behaviour on susceptible and resistant South African wheat genotypes. By making an aphid and the plant substrate into which it is probing (penetrating its stylets, secreting saliva and feeding) part of an electronic circuit, it is possible to record certain repetitive or periodic voltage changes that are correlated with specific probing behaviours and plant tissues. The probing behaviour of *Diuraphis noxia* was studied on two susceptible (Betta and Tugela) and two resistant (1684/Tugela and 2199/Tugela) genotypes using a six-hour recording period. *D. noxia* probing on the two resistant lines 1684/Tugela and 2199/Tugela, spent significantly longer time in pathway activities. The total time of phloem activities was significantly shorter on 1684/Tugela than on susceptible Betta and Tugela while the total phloem time of those aphids feeding on 2199/Tugela was intermediate to the other lines. A significantly shorter duration of E12 periods and E2 fractions was recorded on both resistant lines and *D. noxia* feeding on these lines took significantly longer to attain sustained phloem feeding than those on the susceptible genotypes. Data collected in this study indicates that it is possible to characterise probing behaviour of *D. noxia* on different susceptible and resistant genotypes using the EPG technique. Although significant differences were recorded between genotypes it appears that the six-hour EPG recording is inadequate and an eight-hour EPG recording is suggested for further studies to ensure that all aspects of the probing behaviour of *D. noxia* are fully recorded.

Keywords: *Diuraphis noxia*, electrical penetration graph (EPG), probing behaviour, host plant resistance

Introduction

The Russian wheat aphid *Diuraphis noxia* (Kurdjumov) has had a major impact on the South African wheat industry annually since the early 1980's. Most of the wheat produced in South Africa is grown in the summer rainfall region of the Free State where *D. noxia* is the most serious pest. Symptoms of *D. noxia* infestation on susceptible plants are distinct white, yellow and purple to reddish-purple longitudinal streaks and severe rolling of the leaves of infested plants (Walters, Penn, Du Toit, Botha, Aalbersbeg, Hewitt and Broodryk, 1980). The aphids are found mainly on the adaxial surface of the newest growth, in the axils of leaves or within rolled leaves. Tillers of young plants become prostrate under heavy infestations and at later growth stages ears become

trapped in the rolled flag leaf. *D. noxia* infestation leads to a drastic reduction in chlorophyll content (Kruger and Hewitt, 1984) which, when combined with leaf rolling, causes a considerable loss of effective leaf area on susceptible plants (Walters *et al.,* 1980). Burd and Burton (1992) showed that *D. noxia* infestation resulted in water imbalances in the host plant, expressed as loss of turgor and reduced growth leading to substantial reductions in biomass. Through studying leaf sections of infested, susceptible wheat (cv Adamtas), Matsiliza (2003) showed that typical of most aphids, *D. noxia* probes the leaf between epidermal cells or through the stomata and proceeds on an intercellular pathway through the mesophyll cells to the vascular tissue. Once the vascular bundles are accessed the pathway is intracellular, near and inside the bundle and feeding aphids can act as local sinks, once their stylets have penetrated the functional phloem. *D. noxia* fed preferentially from thin-walled sieve tubes in sink as well as source leaves of wheat and the small longitudinal bundles were preferred (Matsiliza, 2003). By feeding on minor rather than major veins the aphid has the advantage of a shorter pathway to the sieve tubes, less sclerenchyma to impede the passage of the stylets and a food source, which may be richer in both sugars and proteins as the smaller veins have been implicated in the loading and unloading of assimilates. Botha and Matsiliza (2004) reported that *D. noxia* infested leaf tissue was heavily callosed, with callose deposited between the plasma membrane and the cell wall, not only within the phloem tissue, but also in neighbouring vascular parenchymea cells. Deposition of wound callose was found to have disrupted phloem transport and the export of photo-assimilate from the leaves, which could contribute to the characteristically severe yield losses caused by this aphid (Botha and Matsiliza, 2004). Apart from causing substantial yield losses, *D. noxia* has also prevented the planting of spring wheat in the summer rainfall region of South Africa (Du Toit and Walters, 1984; Du Toit 1992) and the utilisation of wheat cultivars with host plant resistance to this pest is seen as the most sustainable and effective solution to the problem.

The ability of an insect to successfully utilise a specific host plant depends on a number of factors including; locating the host determining its suitability initiating feeding and throughout this process evading host defences (Felton and Eichenseer, 2000; Finch and Collier, 2002). This is a very complex process, mediated by various chemical signals and mechanisms that are specific to each individual insect-host combination. In order to fully utilise host plant resistance as a control measure in commercial food crops, a thorough knowledge of the influence of the resistance on the behaviour and fitness of the pest is required. Furthermore, by identifying host genotypes with different influences on the pest insect, a broader base of resistance can be utilised leading to more sustainable / long term control.

Study of the feeding of piercing-sucking insects like homopterans, is difficult because once the insect inserts its stylets into the plant tissue, relevant behaviours occur within the opaque food substrate and are not directly observable (Walker, 2000). Homopteran probing can, however, be effectively studied using the electrical penetration graph (EPG) technique (McLean and Kinsey, 1964; Tjallingii, 1978, 1988; Tjallingii and Hogen Esch, 1993). This method is increasingly being used to study aphid–plant interaction, particularly on resistant host plants, which are deployed for

the control of agriculturally significant pests (Van Helden and Tjallingii, 2000; Walker, 2000). EPG's provide the opportunity of localising the resistance mechanism in the plant tissues (phloem, cuticle, epidermis, mesophyll) and the impact of their mechanical or chemical properties may be derived (Van Helden and Tjallingii, 2000).

This technique works by connecting the aphid and the plant substrate into an electric circuit, which is completed when the aphid stylet penetrates the plant to feed (Figure 3.1). A thin gold wire (8-20 µm in diameter) is glued to the insect's dorsum using water based conductive silver glue. Previous studies (Tjallingii, 1986; Annan, Schaefers, Tingey and Tjallingii, 1997) have shown that this does not influence feeding behaviour significantly if correctly attached. A second electrode is connected to the plant, or plant substrate. A small voltage (either AC or DC depending on the system used) is applied across the insect and the substrate. Completion of the circuit occurs when aphid stylets penetrate the plant, the current flows and a signal can be recorded. Changing electrical properties of the insect-substrate in the circuit, cause a fluctuating current in the circuit, which is converted to voltage fluctuations at the measuring point (Figure 3.1). The recorded and amplified signal is the EPG. Two EPG systems have been used. The first developed, an AC system (McLean and Kinsey, 1964) only records the voltages due to fluctuating electrical resistance (R) over time. The DC system (Tjallingii, 1988), developed later, also records electromotive forces (emf), which are actively generated potentials. Components, R and emf, both reflect important biological information of probing activities.

Certain repetitive or periodic voltage changes (waveforms) have been correlated with specific behaviours (probing, salivation and ingestion) and with the penetration of certain plant tissues (Kimsey and McLean, 1987). As systems have been improved new waveforms and details could be correlated with previously unknown probing activities (Tjallingii, 1988; 2000). Figure 3.2 illustrates a one-hour overview of the main features of a DC EPG, some commonly used parameters and detailed waveforms recognised during plant penetration by aphid stylets. Details of the waveform features and their associated plant tissue and aphid activity are shown in Table 3.1.

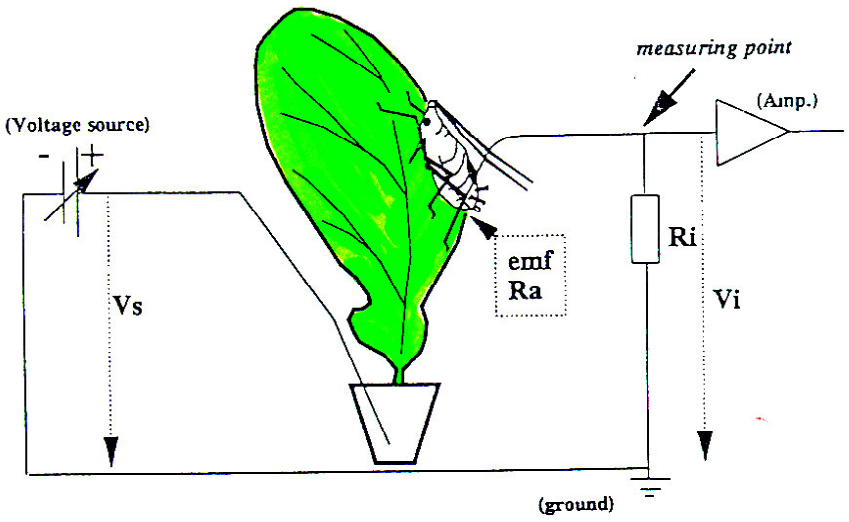

FIGURE 3.1 The primary circuit for EPG recording (DC system). Solid lines represent the primary circuit; dashed lines represent different voltages. The adjustable source voltage (Vs) is introduced into the soil. The penetrating insect is attached to a very thin gold wire electrode by silver paint. The circuit is grounded between the input resistor (Ri) and the voltage source, thus completing the circuit. The amplifier, connected at the measuring point, does not influence the primary circuit. The voltage across Ri (Vi) is the actual signal (EPG) that is measured by the monitoring system after amplification (after Tjallingii, 1996).

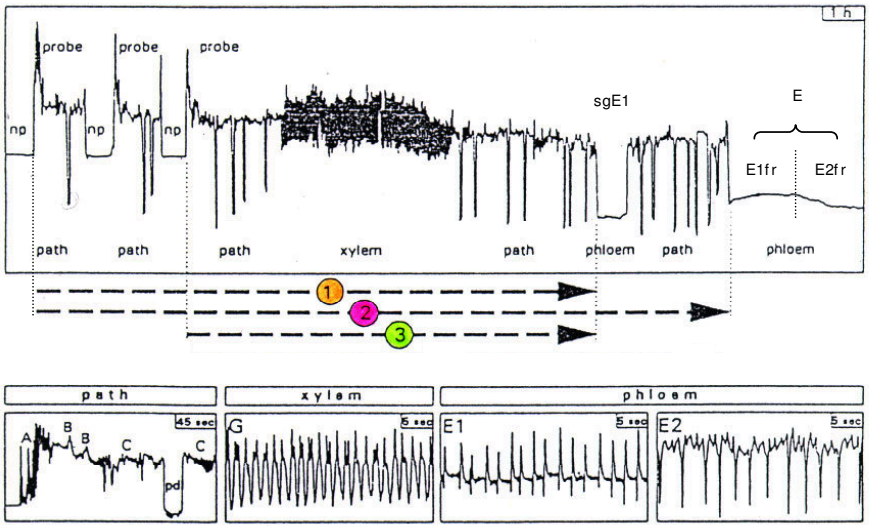

FIGURE 3.2 Electrical penetration graph (EPG) of an aphid. *Top trace*, overview of the main features: np, non probing; probe, period of stylet penetration; path, pathway phase; xylem, xylem ingestion phase; phloem, phloem sieve element phase. *Parameters* (arrows), (1) time to the first sieve element puncture from the beginning of the experiment; (2), time to sustained sieve element ingestion from the beginning of the experiment; (3), time to the first sieve element puncture from the beginning of the probe. *Bottom traces*, detailed waveforms of each phase in the top trace (after Tjallingii, 1996).

Two distinct voltage levels in EPG's are related to stylet tip positions, a high extracellular and a low intracellular voltage level (Figure 3.2). In the EPG non-penetration (non-probing [np]) and three probing/waveform phases namely path(way), xylem and phloem phase, can be distinguished. Pathway phase includes waveforms A, B, C and pd (potential drops), which are usually lumped as waveform or pattern C since no strict separation between A, B and C can be made. Xylem phase includes only waveform G denoting active ingestion from xylem. Phloem phase comprises waveform E1, intracellular sieve element salivation and waveform E2, passive ingestion from the sieve element with concurrent watery salivation. Waveform E1e, possibly represents extracellular watery salivation (see E1) but its actual aphid activity is not yet fully understood. Waveform E1e has not been assigned to any probing phase as yet, this also holds for waveform F. Waveform F represents derailed stylet mechanics, a mechanical 'error' impeding the stylets forming a properly functioning bundle (Tjallingii, 1987).

TABLE 3.1. Waveform features and correlations on EPG. Amplitude (minimum and maximum) relative to waveform A (=100%); repetition rate of peaks or waves in Hertz; voltage level as extracellular (e) or intracellular (i); and the main electrical origin as resistance (R) or electromotive force (emf) (after Tjallingii, 1996; Prado, 1997).

EPG waveform		Features				Correlations to:	
		relative amplitude	frequency / rep. rate	voltage level	electrical origin	Plant tissue	Aphid activity
A		100	5-10	e	R	epidermis	electrical stylet contacts, on/off
B		75	0.2-0.3	e	R	epidermis mesophyll	sheath salivation
C		30	0.2-0.3	e	R	all tissues	activities during stylet pathway
Pd		-	0.02	i	emf	any living cell	intracellular puncture
E1		-	2-4	i	emf	sieve elements	sieve element salivation
E1e		-	2-4	e	emf	extracellular	puzzling E1 incident
E2	p	5	0.5-4	i	R	sieve elements	watery salivation
	w	-	4-7	i	emf	sieve elements	(passive) ingestion – "feeding"
F		5	11-18	e	R emf	all tissues	derailed stylet mechanics
G	w	0-60	4-6	e	emf	xylem	(active) ingestion – "drinking"
	p		1-6	e	R	xylem	unknown subactivity

The EPG is analysed and the beginning of each new waveform is recorded in a spreadsheet. Various calculations are then made in terms of the duration and sequence of the waveforms. These EPG parameters can be classified into two types namely non-sequential and sequential parameters. The non-sequential parameters are independent of any sequence in the probing behaviour of the aphid while sequential parameters are related to a specific sequence as recorded in EPGs (Mayoral, Tjallingii and Castañera, 1996). Non-sequential parameters include total number

of probes, total probing or non-probing time, total pathway time, total time of phloem phase [E1 + E12], total time of phloem ingestion, % aphids showing a period of sustained phloem feeding defined as an E2 period of > 10 minutes [sE2]. In some cases, for parameters where the duration of a specific waveform period is measured, the mean, minimum or maximum duration is reported. Sequential parameters reported include the number of probes < 3min prior to the first phloem phase with the sieve elements [N3m1E1], time to the first phloem phase (E1) in the experiment [T1E1ex], time to first E1 in probe [T1E1pr], time to the first sustained phloem feeding (E2 > 10 min) in the experiment [T1sE2ex], percentage time spent in E2 after the first sustained phloem feeding [E2ind] as well as the number of probes after the first sustained phloem feeding [E2Prind].

Various authors have utilised the EPG technique (both AC and DC systems) as a tool for investigating the probing behaviour of *D. noxia* (Girma, Wilde and Reese, 1992; Kindler, Greer and Springer, 1992; Webster, Porter, Baker and Mornhinweg, 1993; Burd, Elliott and Reed, 1996; Givovich and Niemeyer, 1996; Mayoral *et al.*, 1996; Ni and Quisenberry,1997; Brewer and Webster, 2001). Most EPG studies conducted previously showed that resistance to *D. noxia* manifests in an effect on the phloem activities of the aphids. This study was undertaken to determine the potential of the DC EPG technique to accurately quantify the influence of various resistant genotypes on the probing behaviour of South African Russian wheat aphid in an attempt to differentiate between different resistance genes in South African wheat genotypes.

Material and methods

Plants

D. noxia susceptible Betta (KLEIN IMPACTO) [PI 591916] and Tugela (KAVKAZ/JARAL) [PI 634771] as well as two resistant, advanced breeding lines namely SA1684/4*Tugela and SA 2199/4*Tugela containing the resistance *ex* SA 1684 [PI 137739 (Dn1)] and SA 2199 [PI 262660 (Dn2)] (Du Toit, 1987, 1988, 1989a, 1989b) respectively, were used in the study. These lines will be referred to as 1684/Tugela and 2199/Tugela throughout. Sufficient plants were prepared for each genotype for the duration of the study with three seeds sown per pot (15cm diameter) containing soil previously mixed with fertiliser. Plants were maintained in a greenhouse at 16°C night : 24°C day with natural light until they were used in the experiment.

Aphids

Aphids used in the EPG experiment were the progeny of a single, apterous *D. noxia*. This colony was maintained in a separate cage in the greenhouse chamber where the main colony was kept. Fourth or fifth instar, apterae *D. noxia* were collected early in the morning from the colony by carefully brushing them from the leaves into a Petri dish containing a filter paper disc. Aphids were brought to the laboratory where they were attached to 20µm diameter gold wire with water soluble, conductive silver glue, under a compound microscope.

EPG set-up

The experiment was performed over an 8-month period in 2000 at the ARC-Small Grain Institute in Bethlehem, South Africa. For each EPG run two pots of each of the four entries (plants approximately two weeks old when used) were moved from the greenhouse to the laboratory, where they were randomly assigned a position in the Faraday cage needed to shield recording from noise. The preferred probing site of *D. noxia* is known to be the adaxial surface of the newest growth, in the axils of leaves or within rolled leaves (Walters *et al*., 1980); therefore, the distal third of the youngest leaf was clamped in a flat position facing upward using a hairclip covered in masking tape to prevent bruising the leaf, as illustrated in Figure 3.3. Approximately one hour after collection in the greenhouse the wired aphids were randomly assigned to the eight available channels and the experiment was started. Figure 3.4 shows the EPG set-up including plants and the GIGA-8 (constructed by Wageningen University, The Netherlands) with eight first stage amps inside a Faraday cage. A thermograph recorded temperature throughout.

FIGURE 3.3 Close-up of leaf held in position by hairclip and EPG probe with *D. noxia* wired in position.

FIGURE 3.4 EPG set-up: GIGA-8 with eight channels, the plants and the thermograph used to record temperature within the Faraday cage.

Approximately 20 replicates (each replicate with a new aphid and plant) were collected for each of the four genotypes. Six-hour (360 min; 21600s) EPG recordings were made and the graphs were analysed using Stylet 3.0 software developed by Tjallingii in ASYST ™ (STYLET 3.0, 1999). This software is used to work though the recorded waveforms and note the beginning of each waveform as well as the voltage at the starting point. Aphids falling off the leaf, or becoming detached from the gold wire were not included in the analysis. Data were prepared for statistical analysis with Microsoft Excel and analysed using Mann-Whitney-U test at p=0.05 (Van Ark, 1992). Comparisons were made of Betta vs Tugela, Tugela vs 1684/Tugela, Tugela vs 2199/Tugela and 1684/Tugela vs 2199/Tugela.

EPG waveforms and parameters.

The waveforms E1 and E2 can sometimes collectively be referred to as E (Tjallingii, 1996). A period of E1 may be followed by E2, referred to as an E12 period, in which E1 and E2 will then be referred to as fractions (E1fr and E2fr), whereas E1 without a subsequent E2 is referred to as a single E1 (sgE1). Usually a short period of E1 is followed by an E2 period. In some instances, the two patterns can occur simultaneously in which case ambiguous mixtures are classified as E1 and only clear E2 patterns classified as E2 (Van Helden and Tjallingii, 1993). The parameters considered here are listed below. All parameters are means per aphid or maximum values among all aphids.

1. Duration of the 1st probe
2. Sum of probing, i.e. all time stylets are in the plant tissue
3. Sum of pathway including waveforms A, B and C
4. Sum of phloem (sgE1 + E12)
5. Sum of single E1 periods
6. Sum of E12 periods
7. Sum of E1 fractions
8. Sum of E2 fractions
9. Sum of F/G
10. Mean duration of single E1 periods
11. Mean duration of E12 periods
12. Mean duration of E1 fractions
13. Mean duration of E2 fractions
14. Number of probes
15. Number of path periods
16. Number of single E1 periods
17. Number of E12 periods
18. Number of E1 fractions
19. Number of E2 fractions
20. Number of F/G periods
21. Maximum duration of single E1 period
22. Maximum duration of E12 period
23. Maximum duration of E1 fraction

24. Maximum duration of E2 fraction
25. Number of probes shorter than 3min before 1st E
26. Time to 1st E1 in experiment, i.e. from start of 1st probe
27. Time to 1st E1 in probe
28. Time to 1st sustained E2 (>10 min) in experiment, i.e. from start of 1st probe
29. % Time in E2 after 1st sE2
30. Number of probes after 1st sE2
31. % Aphids with sE2
32. Total number E1 periods followed by F or G periods

Results

Table 3.2 shows there were no differences in the duration of the first probe or the sum of all probing on the four entries. The mean number of probes and path periods did not differ between genotypes either.

D. noxia clearly spent longer time in pathway activities on the two resistant lines 1684/Tugela and 2199/Tugela and less time in the phloem on 1684/Tugela than on susceptible Betta and Tugela. Total phloem time on 2199/Tugela was intermediate to the other lines (Figure 3.5). A similar situation holds for the maximum duration of E12 periods and E2 fractions but no significant differences for the mean duration of the various phloem periods were found between the four genotypes (Table 3.2).

The sum of E12 periods was significantly ($p<0.05$) shorter on the resistant lines than on the susceptible (Figure 3.6), while the sum of E2 fractions was shorter on 1684/Tugela than on Betta and Tugela, with that on 2199/Tugela intermediate. No significant differences occurred between genotypes for the sum of all single E1 periods or the sum of all E1 fractions. Remarkably, an E1 period was followed by an F or G period considerably more often on the resistant than on the susceptible plants (Figure 3.7). This was also apparent in the percentage of the total number of aphids showing an F or G period after an E1 period with Betta = 0; Tugela = 0; 1684/Tugela = 9.9% and 2199/Betta = 6.6%. The frequency tested significantly different using a one-sample Chi^2 test (Van Ark, 1992) with $Chi^2=10.317$, df=3, $p=0.05$, Tabled $Chi^2=7.815$.

TABLE 3.2 EPG parameters recorded for *D. noxia* feeding on susceptible and resistant South African wheat genotypes. Total duration of recordings was 6h (= 21600 sec). All figures represent mean values, when followed by a different letter entries within a row differ significantly (Mann-Whitney U-test, $p < 0.05$).

Parameter	Betta n=23	Tugela n=20	1684/Tugela n=21	2199/Tugela n=22
Duration of 1st probe (sec)	1236 a	177 a	240 a	135 a
Sum of all probing (sec)	16321 a	15985 a	16524 a	16974 a
Number of probes	10.1 a	11.3 a	13.4 a	11.0 a
Number of path periods	16.2 a	15.9 a	18.7 a	16.5 a
Number of single E1 periods	3.1 a	2.1 a	2.9 a	3.1 a
Number of E12 periods	2.2 a	2.5 a	1.4 b	1.4 b
Number of E1 fractions #	2.2 a	4.8 b	1.7 ab	1.6 a
Number of E2 fractions	2.2 a	2.7 a	1.5 b	1.4 b
Number of F/G periods *	1.3 a	0.7 a	1.9 ab	1.7 b
Mean duration single E1 period (sec)	267 a	312 a	225 a	188 a
Mean duration E12 period (sec)	3932 a	4097 a	2758 a	3703 a
Mean duration E1 fraction (sec)	377 a	463 a	379 a	560 a
Mean duration E2 fraction (sec)	3493 a	3528 a	1841 a	3543 a
Maximum duration of E1 period (sec)	398 a	421 a	387 a	361 a
Maximum duration of E12 period (sec)	4758 a	5593 a	2860 b	3556 ab
Maximum duration of E1 fraction (sec)	523 a	639 a	514 a	730 a
Maximum duration of E2 fraction (sec)	4271 a	4910 a	2376 b	3523 ab
Number probes < 3 min before 1st E1	37 b	76 a	67 a	68 a
% Time in E2 after 1st sE2	57 a	60 a	48 a	60 a
Number of probes after 1st sE2	3.24a	2.00 a	4.00 a	1.43 a

Betta vs Tugela: Mann Whitney U-value = 84.5, Z=3.543 p=0.0047; *Tugela vs 1684/Tugela*: Mann-Whitney U-value = 54, Z=4.069 ns; *Tugela vs 2199/Tugela*: Mann Whitney U-value = 48.52, Z=4.319 ns; *1684/Tugela vs 2199/Tugela*: Mann-Whitney U-value = 217.5, Z=-0.328 ns

* *Tugela vs 1684/Tugela*: Mann Whitney U-value = 148.5, Z=-1,693 ns; *Tugela vs 2199/Tugela*: Mann-Whitney U-value = 122, Z=-2.616, p= 0.009

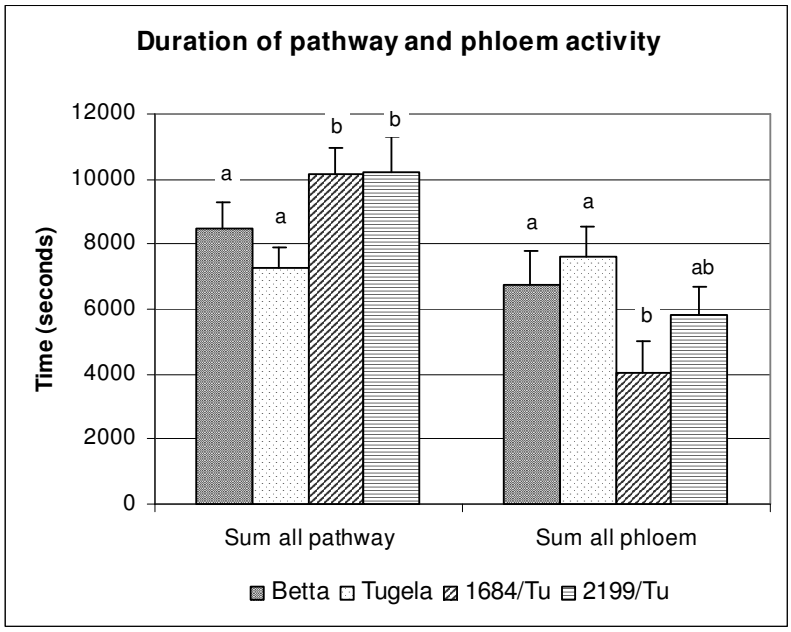

FIGURE 3.5 Sum of all pathway and phloem activities (sgE1 + E12) for *D. noxia* probing on susceptible (Betta and Tugela) and resistant (1684/Tugela and 2199/Tugela) genotypes. (^{abc} parameter means without letters in common differ significantly, Mann-Whitney U-test, $p < 0.05$)

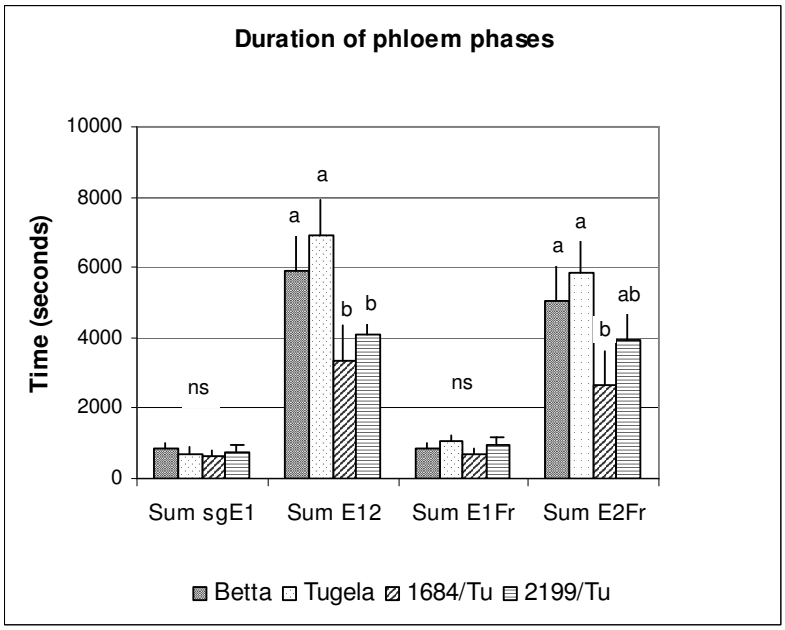

FIGURE 3.6 Sum of all phloem phases for *D. noxia* probing on susceptible (Betta and Tugela) and resistant (1684/Tugela and 2199/Tugela) genotypes. (^{abc} parameter means without letters in common differ significantly, Mann-Whitney U-test, $p < 0.05$)

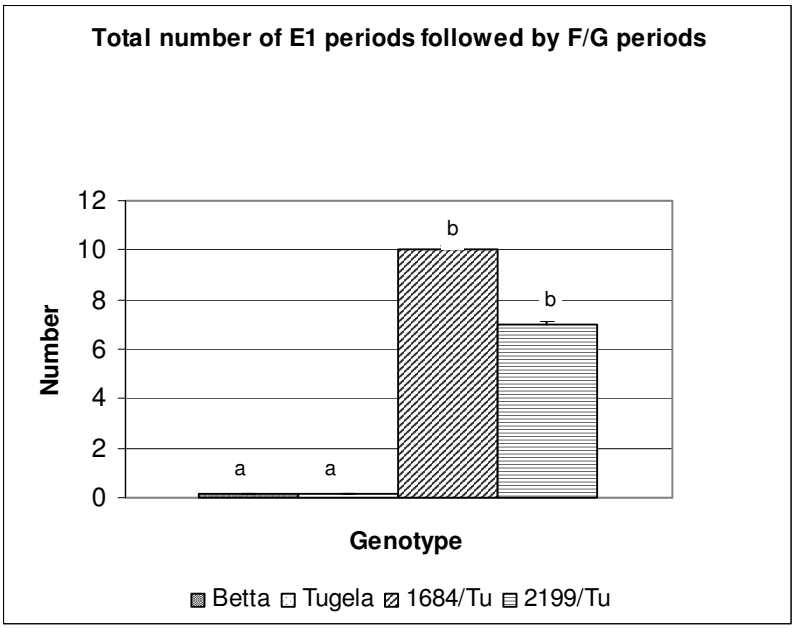

FIGURE 3.7 Total number of E1 periods followed by a For G period for *D. noxia* probing on susceptible (Betta and Tugela) and resistant (1684/Tugela and 2199/Tugela) genotypes. (^{abc} means without letters in common differ significantly, Mann-Whitney U-test, $p< 0.05$)

In Figure 3.8 the sequential parameters are shown for the time taken by *D. noxia* on the various genotypes to reach the sieve elements and thereafter to attain sustained phloem feeding. No differences were noted between genotypes for the time needed to reach the sieve elements in either the experiment (T1E1ex), or the probe (T1E1pr). The time to sustained phloem feeding in the experiment (T1sE1ex) was delayed on resistant 1684/Tugela and 2199/Tugela suggesting constraints of phloem sap acceptance. No differences were noted between genotypes for the number of probes after sustained phloem feeding, or the percentage time in E2 subsequent to the 1st sE2 period (Table 3.2). A one sample Chi^2 test (Van Ark, 1992) found the frequency of aphids with a sustained E2 period to be different between genotypes (Betta = 65%, Tugela = 90%, 1684/Tugela = 52%, 2199/Tugela = 59%; Chi^2=12.12, df=3, p=0.05, Tabled Chi^2=7.815) with the resistant lines having fewer aphids reach sustained phloem ingestion than on susceptible cultivars.

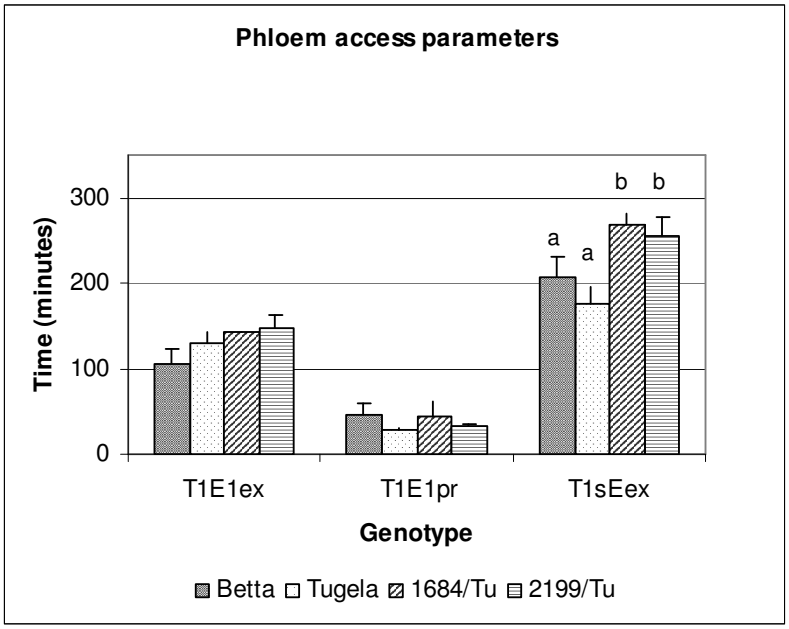

FIGURE 3.8 Sequential parameters reflecting phloem access and acceptance by *D. noxia*. The time to 1st E1 in the experiment (T1E1ex), time to 1st E1 in the probe (T1E1pr) suggest equal mesophyll and initial phloem properties. (^{abc} parameter means without letters in common differ significantly, Mann-Whitney U-test, $p < 0.05$)

Discussion and conclusions

Data collected in this experiment confirms that the EPG technique can highlight differences between probing behaviour of *D. noxia* on various wheat genotypes. This data appears to support reported data from previous studies (Girma *et al*., 1992; Kindler *et al*., 1992; Webster *et al*., 1993; Burd *et al*., 1996; Givovich and Niemeyer, 1996; Mayoral *et al*., 1996; Ni and Quisenberry,1997; Brewer and Webster, 2001) that the effect of resistance is reflected in the phloem activities of *D. noxia*.

Resistant 1684/Tugela appears to exert a stronger negative influence on *D. noxia* than 2199/Tugela, although this difference is not significant. The occurrence of many F and G periods after a period of E1 on resistant lines may indicate some difficulty for *D. noxia* in attaining phloem ingestion (E2). Tjallingii (1988) reported that no transitions to E (phloem ingestion) had ever been recorded from waveform F and that no sap exudation had been found from stylets severed during this waveform. Furthermore, electron micrographs of stylet pathways during F showed the stylets occurring in the cell walls. The time to sustained phloem feeding in the experiment (T1sE1ex) was

delayed on both 1684/Tugela and 2199/Tugela, suggesting constraints of phloem sap acceptance on the resistant genotypes when compared to the susceptible genotypes. Aphids probing on the resistant lines were only able to attain sustained phloem feeding more than 250 minutes after the start of the experiment. This is in the fourth hour of the six-hour duration of the experiment (total duration 360 minutes) conducted in this study. It is possible that the duration of the experiment was not long enough to fully characterise the probing behaviour on the resistant genotypes. It is therefore suggested that eight-hour recordings be made in future so that all aspects of the probing behaviour of the *D. noxia*, in particular the activities within the vascular bundles, are fully recorded.

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Chapter 4

Constitutive and Induced Host Plant Resistance: EPG measurement of Russian wheat aphid, *Diuraphis noxia,* probing behaviour on noninfested and preinfested plants.

Abstract

The Russian wheat aphid, *Diuraphis noxia* (Kurdjumov), is an exceptionally damaging pest of cultivated wheat (*Triticum aestivum*). Resistant cultivars have been developed and deployed in South Africa and the USA for control of this pest, but the exact influence of resistance on the aphid is not yet understood. *D. noxia* susceptible Tugela (KAVKAZ/JARAL) [PI 634771] and a near isogenic *D. noxia* resistant line, TugelaDn [PI 591932] were used in EPG study, with a DC system, to determine the effect of host plant resistance on *D. noxia* probing. Susceptible and resistant genotypes were each subjected to four pre-conditioning treatments (no preinfestation, preinfested for one day, preinfested for three days, preinfested for five days) prior to being used. The effect of resistance in TugelaDn on the probing behaviour of *D. noxia* appears to be primarily phloem related with no epidermal, mesophyll or vascular parenchyma factors involved in the resistance. Constitutive resistance is characterised by a significantly longer time needed to attain sustained phloem ingestion on the resistant genotype, more F/G periods, a longer total duration of these waveforms, a higher number and sum of single phloem salivation periods and a shorter mean duration and sum of phloem ingestion. More phloem salivation fractions, shorter than one minute, occur on previously uninfested resistant plants in comparison to uninfested susceptible plants. Induced resistant TugelaDn is characterised by a smaller sum of phloem activities on plants preinfested for one and five days, a shorter E12 sum and mean duration on plants preinfested for one and five days, and a shorter mean and sum of phloem ingestion on plants preinfested for one and five days. *D. noxia* feeding on five day induced resistant plants showed more E2 fractions shorter than ten minutes and fewer E2 fractions longer than 60 minutes indicating a reduction in the ingestion of nutrients. In the case of both constitutive and induced resistance, initiation of phloem ingestion appears easier on susceptible Tugela with a higher number of single phloem salivation periods on the resistant genotype. Reduced ingestion of phloem sap, as shown by this EPG study, is consistent with the mechanisms of resistance reported in previous studies for this line. It is suggested that for future EPG studies all entries be infested for 5 days prior to being used as this treatment gave the most comprehensive indication of the host plant resistance to *D. noxia.*

Keywords: constitutive resistance, induced resistance, *Triticum aestivum,* Tugela, TugelaDn

Introduction

The Russian wheat aphid, *Diuraphis noxia* (Kurdjumov), is an exceptionally damaging pest of cultivated wheat (*Triticum aestivum*) and has been known to occur in South Africa since 1978 (Walters, Penn, Du Toit, Botha, Aalbersberg, Hewitt, and Broodryk, 1980). The discovery of bread wheat accessions with resistance to *D. noxia* by Du Toit (1987; 1988; 1992), led to a resistance breeding programme and the first resistant commercial cultivar, TugelaDn, was released in 1992 in South Africa (Van Niekerk, 2001). To date resistant cultivars have been developed and released in South Africa (Tolmay and Van Deventer, 2005) and the USA in Colorado (Randolph, Peairs, Koch, Walker, Stubbs, Quick, and Haley, 2005) and Kansas (Qureshi, Jyoti and Michaud, 2005).

The electrical penetration graph (EPG) technique (McLean and Kinsey, 1964; Tjallingii, 1978, 1988; Tjallingii and Hogen Esch, 1993) is often used to study the probing behaviour of aphids. It gives valuable information regarding the insect–plant interaction, which is particularly important in the investigation of resistant host plants, which are being exploited as an environmentally responsible approach for the control of agriculturally significant pests (Van Helden and Tjallingii, 2000; Walker, 2000). EPG's provide the opportunity of localising the resistance mechanism in the plant, be it mechanical or chemical properties of plant tissues (phloem, cuticle, epidermis, mesophyll) (Van Helden and Tjallingii, 2000). Electronic monitoring of *D. noxia* probing has been reported on wheat, oats, rye (hosts) and sorghum (nonhost) by Girma, Wilde and Reese (1992), on susceptible wheat as well as resistant and susceptible slender wheatgrass accessions by Kindler, Greer and Springer (1992), on barley by Webster, Porter, Baker and Mornhinweg (1993), on insecticide treated wheat by Burd, Elliott and Reed (1996), on resistant wheats and triticales by Givovich and Niemeyer (1996), on bread wheat, hard wheat, triticale, rye and barley with different hydroxamic acid levels by Mayoral, Tjallingii and Castañera (1996), on *D. noxia* resistance donor accessions by Ni and Quisenberry (1997a) and on water stressed, *D. noxia* resistant barley by Brewer and Webster (2001).

Most EPG studies conducted previously show that resistance to *D. noxia* manifests in an effect on the phloem feeding of the aphids. Girma *et al*. (1992) reported that *D. noxia* showed more pathway and less phloem phase activities on a non-host and that it took four times longer to show the first phloem activities and achieve committed phloem ingestion while there were no differences in phloem phase activity on the host wheat, rye and oat plants. Kindler *et al*. (1992) reported longer phloem phase activity on the most susceptible wheatgrass accession and an increase in non-probing behaviour on the resistant wheatgrasses. A higher frequency of periods of non-probing, pathway and other non-phloem activity on resistant barley lines was reported Webster *et al*. (1993) with *D. noxia's* stylet tips spending more time in the phloem on susceptible lines. *D. noxia* on resistant lines took longer to reach the first period of phloem activity longer than 15 min in length (called committed phloem 'ingestion', although not clear in the AC EPGs, see below) on resistant barley lines. Burd *et al*. (1996) reported that *D. noxia* on untreated susceptible wheat spent ≈ 35% of the total time in stylet pathway activities with no differences in probing behaviour being observed

between Gaucho-treated and untreated wheat. Ni and Quisenberry (1997b) reported that *D. noxia* probed more diurnally than nocturnally, and that diurnal probes were shorter than nocturnal ones. No significant differences were reported between the resistance donors PI 137739 and PI 262660, however, total probing duration, duration per probe and duration of salivation (pathway activities) and ingestion per probe was lower on PI 137739 than on the susceptible control Arapahoe wheat. In contrast PI 262660 did not differ from Arapahoe. It was also reported that *D. noxia* seemed to feed more nocturnally on PI 137739 and Halt, possibly to compensate for less diurnal feeding (Ni and Quisenberry,1997b). Brewer and Webster (2001) reported that *D. noxia* took longer to first enter the sieve element phase of probing and that the duration of the sieve element phase on resistant 'STARS-9301B' barley was shorter than on susceptible 'Morex'. The studies referred so far all used AC EPGs in which no distinction can be made within the phloem phase activities between phloem (sieve element) salivation and ingestion. The DC EPG system allows this distinction and in two DC EPG studies higher levels of hydroxamic acid in test entries led to a delay in attaining sustained phloem feeding (Givovich and Niemeyer, 1996), less probing and a lower percentage of aphids reaching sustained phloem ingestion (Mayoral *et al*., 1996).

TugelaDn and Tugela, the near-isogenic, susceptible cultivar from which TugelaDn was developed, have been used in numerous studies to elucidate the nature of the resistance ex donor accession PI 137739 (Bahlmann, Govender and Botha, 2003; Botha, Nagel, Van der Westhuizen and Botha, 1998; Mohase and Van der Westhuizen, 2002; Tolmay and Van Deventer, 2005; Van der Westhuizen and Botha, 1993; Van der Westhuizen and Pretorius, 1995, 1996; Van der Westhuizen, Qian and Botha, 1998a, 1998b; Van der Westhuizen, Qian, Wilding and Botha, 2002; Wang, Quisenberry, Ni and Tolmay, 2004a; Wang, Quisenberry, Ni and Tolmay, 2004b). The availability of detailed information on the biochemistry, structural botany as well as mechanisms of resistance and influence of the resistance on *D. noxia* under field conditions makes these lines unique and valuable in the ongoing endeavour to understand and describe exactly how and why resistance to *D. noxia* works. This study aims to: a) describe the probing behaviour of *D. noxia* on susceptible Tugela and resistant TugelaDn using the DC EPG system, thus enabling a better understanding of the phloem (sieve element) salivation and ingestion; and b) to investigate the influence of possible constitutive and induced components on *D. noxia* probing.

Material and Methods

Plants

D. noxia susceptible Tugela (KAVKAZ/JARAL) [PI 634771] and a near isogenic *D. noxia* resistant line, TugelaDn [PI 591932] (Van Niekerk, 2001, Tolmay, Du Toit and Smith, 2006) were planted daily from 2nd January 2001 to 22nd February 2001 in a growth chamber set at 22°C and 24h light to obtain uniform plants. For each cultivar four 15cm diameter plastic pots containing soil previously mixed with fertiliser were prepared and three seeds were planted per pot. Once seedlings had emerged and were approximately 10cm tall, the plants were moved to a greenhouse maintained at

16℃ night : 24℃ day with natural light. Each day two pots of Tugela and TugelaDn were moved to a separate cubicle in the greenhouse, maintained at the same conditions as described previously, where they were infested with approximately 15-20 *D. noxia* of mixed instars from the greenhouse colony which was maintained on the susceptible cultivar Betta.

Aphids

Aphids used in the EPG experiment were the progeny of a single, apterous *D. noxia*. This colony was maintained in a separate cage in the greenhouse chamber where the main colony was kept. Adult apterae were collected early in the morning from the colony by carefully brushing them from the leaves into a Petri dish containing a filter paper disc. Aphids were brought to the laboratory where they were attached to 20µm diameter gold wire with water soluble, conductive silver glue, under a compound microscope.

EPG set-up

Each day, four susceptible and four resistant, two-week old, aphid preconditioned plants (one for each of the eight treatments) to be used in the experiment were transferred from the greenhouse to the laboratory, where they were randomly assigned a position in the Faraday cage. The preferred probing site of *D. noxia* is known to be the adaxial surface of the newest growth, in the axils of leaves or within rolled leaves (Walters *et al.*, 1980). On susceptible plants, using leaf tissue close to the axil proved to be too difficult with leaves being tricky to unroll and easily breaking off. Instead, the adaxial surface of the distal third of the youngest leaf was clamped in a flat position facing upward using a hairclip covered in masking tape to prevent bruising the leaf, as illustrated in Figure 3.3 (Chapter 3) as a standard procedure for all genotypes and treatments. Approximately 1h after collection in the greenhouse the wired aphids were randomly assigned to the eight available channels and the experiment was started. Figure 3.4 (Chapter 3) shows the EPG set-up including plants and the GIGA-8 (constructed by Wageningen University, The Netherlands) with eight first stage amps inside a Faraday cage. A thermograph recorded temperature throughout.

Experimental design and data treatments

Plants used for EPG recordings (started on 15th January 2001) had the following treatments: i) previously uninfested Tugela (Tug-0), ii) Tugela preinfested for one day (Tug-1), iii) Tugela preinfested for three days (Tug-3), iv) Tugela preinfested for five days (Tug-5), by which time damage symptoms were visible, v) previously uninfested TugelaDn (TugDn-0), vi) TugelaDn preinfested for one day (TugDn-1), vii) TugelaDn preinfested for three days (TugDn-3) and viii) TugelaDn preinfested for five days (TugDn-5).

Approximately 20 replicates (each replicate with a new aphid and plant) were collected for each of the eight treatments as described above. Eight-hour (480 min; 28800s) EPG recordings were made and the graphs were analysed using Stylet 3.0 software developed by Tjallingii in ASYST ™ (Tjallingii, 1999). Aphids falling off the leaf or becoming detached from the gold wire were not included in the analysis. Data were prepared for statistical analysis with Microsoft Excel. Data

were analysed for constitutive effects (previously uninfested Tugela vs TugelaDn [Tug-0 vs TugDn-0]) and induced effects (previously infested Tugela vs TugelaDn) using either a univariate analyses with LSD Tuckey to compare means or non-parametric U-Test of Mann Whitney (Van Ark, 1992). Where required, transformations were used to stabilise variances and normalise the data. A statistical analysis of main effects (combined treatments for Tugela and TugelaDn) was performed using the non-parametric U-Test of Mann Whitney (Van Ark, 1992) and the entire data set was subjected to a correlation analysis using NCSS (Hintze, 2004).

Results

Constitutive effects: Tugela vs TugelaDn [Tug-0 vs TugDn-0]

For *D. noxia* probing on previously uninfested Tugela and TugelaDn, there was no significant difference ($p<0.05$) in the sum of all probing, the number of probes, the sum of all pathway activity and the number of path periods (Table 4.1). A Mann Whitney U-test showed no difference between the susceptible and resistant lines for the duration of the first probe, which was 521±170 sec on Tugela, and 1630 ±376 seconds on TugelaDn (Mann-Whitney U-value = 187.0, Z = 0.339, p = 0.74140). The time to the first phloem phase in the experiment as well as within a probe, were similar in both lines (Table 4.1) which also indicates that no epidermal, mesophyll or vascular parenchyma factors of constitutive resistance play a role in TugelaDn. The number of short probes (<3 min) recorded before the first sustained phloem ingestion did not differ between uninfested genotypes. On average two short probes on Tugela were recorded compared to three on TugelaDn (Mann-Whitney U-value = 175, Z = 0.664, p = 0.5156).

When comparing phloem activity there was no difference in the total time spent by *D. noxia* in the phloem tissue (sgE1 + E12) on Tugela and TugelaDn. However, the number of single E1 salivation periods (Figure 4.1) and the sum of the single E1 periods (Figure 4.2) was higher for *D. noxia* probing on TugelaDn. This suggests that the aphids accessed the phloem without switching to phloem ingestion more on resistant plants, implying that on susceptible plants initiation of phloem ingestion (E2) was easier. The mean duration of these single E1 periods did not differ between the two genotypes.

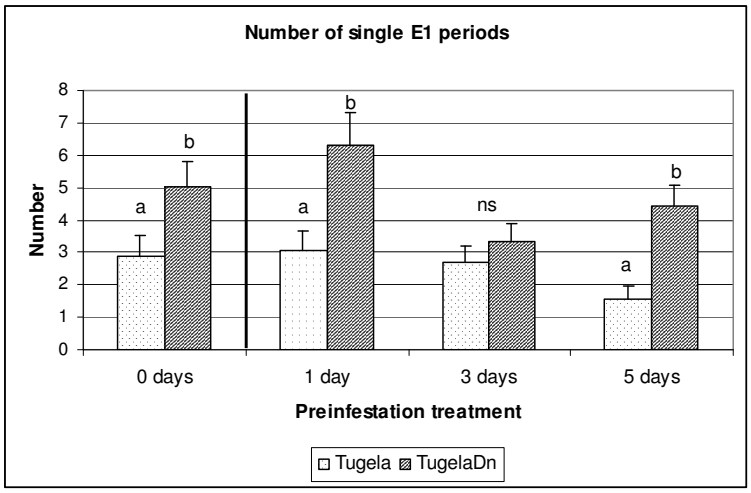

FIGURE 4.1 Number of single E1 periods for *D. noxia* probing on susceptible Tugela and resistant TugelaDn, preinfested for none (constitutive resistance), one, three and five days (induced resistance). (^{abc} means within a preinfestation treatment without letters in common differ significantly, Mann Whitney U-Test ($p<0.05$)).

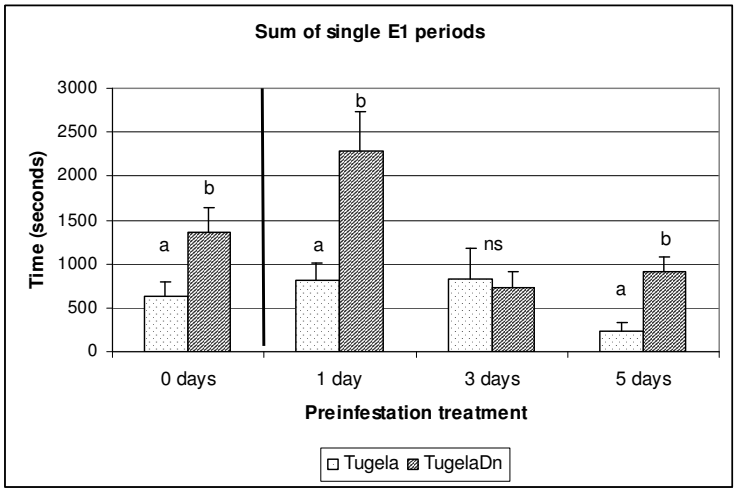

FIGURE 4.2 Sum of single E1 periods for *D. noxia* probing on susceptible Tugela and resistant TugelaDn, preinfested for none (constitutive resistance), one, three and five days (induced resistance). (^{abc} means within a preinfestation treatment without letters in common differ significantly, Mann Whitney U-Test ($p<0.05$)).

When considering E12 periods, no difference was found between the number, duration or sum of the periods between the two genotypes (Table 4.1). Within the E12 periods, no difference was noted regarding the number of E1 fractions, the sum of E1 fractions or the mean of E1 fractions.

TABLE 4.1 Parameters of *D. noxia* probing measured by eight hour EPG for susceptible Tugela (Tug-0) and resistant TugelaDn (TugDn-0)

Parameter	Transf.	Tugela-0 (Susceptible) n=19		TugelaDn-0 (Resistant) n=21		SS Genotype	SS error	*Df*	MS error	F	p
		Mean	***SE***	***Mean***	***SE***						
Sum of probing (sec)	-	25733	617.1031	26325	377.0805	3.43E+06	1.84E+08	37	4.98E+06	0.688	ns
Number of probes	-	8.16	1.3876	10.70	1.6624	6.30E+01	1708.73	37	46.18	1.36344	ns
Sum of pathway	log	3.862	0.051212	3.984	0.037233	1.43E-01	1.4237	37	0.0385	3.73	ns
Number of path periods	-	16.47	2.3624	22.05	2.4328	302.98	4157.69	37	112.37	2.6963	ns
Sum of phloem (sgE1 +E12) (sec)	-	17037	1212.107	14310	1092.816	7.25E+07	9.56E+08	37	2.58E+07	2.8042	ns
Number of single E1 periods	-	2.90	0.607	5.30	0.7438	56.3695	335.99	37	9.08	6.2076	0.01733
Mean duration single E1 periods (sec)	-	165.922	30.80958	244.565	32.71217	60261	731269	37	19764	3.0490	ns
Sum of single E1 periods	log(n+1)	2.118	0.280839	2.951	0.114049	6.7577	31.9164	37	0.8626	7.8341	ns
Number of E12 periods	-	4.63	0.6720	4.45	0.5403	3.21E-01	2.65E+02	37	7.17E+00	0.0448	ns
Mean duration of E12 periods	log	3.64121	0.109039	3.48295	0.084297	0.244	6.7665	37	0.1829	1.334	ns
Sum of E12 periods # (sec)	-	16403	1287.762	12879	1266.017	1.21E+08	1.18E+09	37	3.18E+07	3.8061	ns
Number of E1 fractions	-	6.00	0.78	7.00	0.91	16.88	553.1	37	14.56	0.288	ns
Mean duration of E1 fractions (sec)	-	513	48.93	651	91.71	190592	4380694	37	115281	0.206	ns
Sum of E1 fractions (sec)	-	2753	374.28	3875	479.46	1.3E+07	1.4E+08	37	380617	0.077	ns

Table 4.1 continued: Parameter	**Transf.**	**Tugela-0 (Susceptible) n=19**		**TugelaDn-0 (Resistant) n=21**		**SS Genotype**	**SS error**	***Df***	**MS error**	**F**	**p**
		Mean	***SE***	***Mean***	***SE***						
Number of E2 fractions	-	5.37	0.705909	5.75	0.627757	1.42E+00	320.171	37	8.653	0.1639	ns
Mean duration of E2 fractions	log	3.437	0.121263	3.081	0.109728	1.2358	9.6044	37	0.2596	4.761	0.03554
Sum of E2 fractions (sec)	-	13650	1528.68	8870	1298.88	2.23E+0	1.44E+09	37	3.89E+07	5.7187	0.02198
Number of F/G periods	-	1.05	0.247487	2.55	0.472814	21.8462	105.8974	37	2.8621	7.6330	0.00888
Sum of F/G	log(n+1)	1.639	0.334158	2.549	0.302357	8.07E+00	72.9278	37	1.971	4.0939	0.05031
Time to 1st E1 in experiment	log	3.706	0.043481	3.679	0.055507	0.0071	1.8174	37	0.0491	0.14	ns
Time to 1st E1 in probe	log	3.3344	0.044016	3.2541	0.038702	0.0629	1.2317	37	0.0333	1.89	ns
Time to 1st sustained E2 (E2>10 min) in experiment	log	3.901	0.051884	4.135	0.053106	0.5358	1.9923	37	0.0538	9.95	0.00319
%Time in E2 after 1st sE2	-	0.6763	0.063163	0.5783	0.075854	0.09356	3.55091	37	0.09597	0.9749	ns

Mann-Whitney-U = 132; Z = 1.629659. p = 0.103 Rank sum Tug-0 = 438; Rank sum TugDn-0 = 342.

However, the E2 fractions differed between the genotypes. The mean duration of E2 fractions was significantly shorter on the resistant genotype (Figure 4.3) consequently the E1/E2 ratio was smaller on TugelaDn than on Tugela. The number of E2 fractions did not differ between genotypes but their summed duration was considerably reduced on TugelaDn (Figure 4.4) indicating a phloem related factor of resistance. Also, the longer time from the first probe to the first sustained phloem feeding on TugelaDn (Table 4.1) supports this observation.

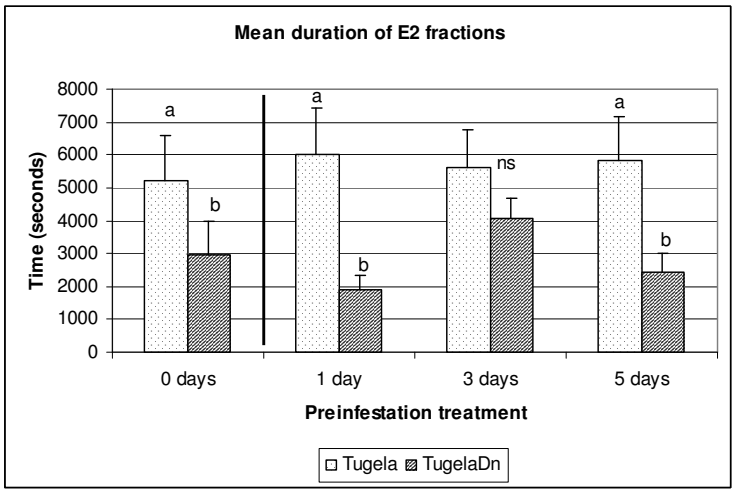

FIGURE 4.3 Mean duration of E2 fractions for *D. noxia* probing on susceptible Tugela and resistant TugelaDn, preinfested for none (constitutive resistance), one, three and five days (induced resistance). (^{abc} means within a preinfestation treatment without letters in common differ significantly, Mann Whitney U-Test ($p<0.05$)).

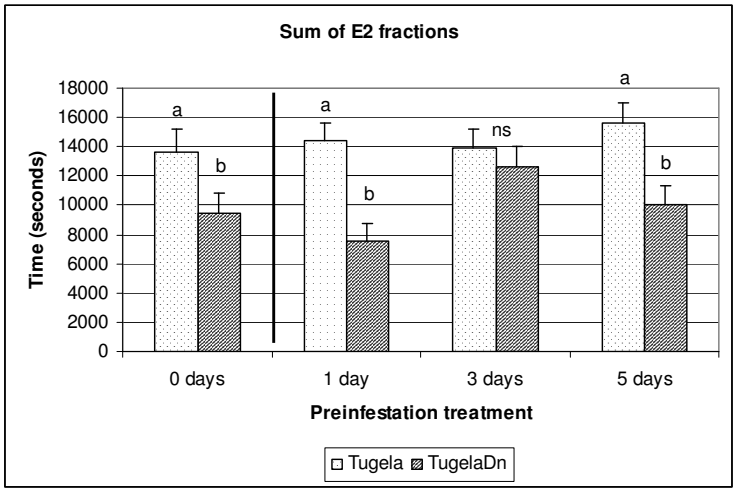

FIGURE 4.4 Sum of E2 fractions for *D. noxia* probing on susceptible Tugela and resistant TugelaDn, preinfested for none (constitutive resistance), one, three and five days (induced resistance). (^{abc} means within a preinfestation treatment without letters in common differ significantly, Mann Whitney U-Test ($p<0.05$)).

Four arbitrary classes were specified to characterise the distribution of E1 fractions for each genotype and to highlight possible differences between the genotypes. *D. noxia* probing on TugelaDn showed more E1 fractions of less than one minute in duration (Table 4.2). Row x Column Chi^2 test showed that observed frequencies of the four classes as such differed statistically significantly between Tugela and TugelaDn (Table 4.3). Medium length E1 periods are thought to suppress phloem protein clotting. The lack of difference between medium length E1 periods, which form the largest proportion of the total, may indicate that these E1 periods are equally effective on both genotypes. In some other cases of phloem based resistance a considerable increase of medium length E1 periods has been associated with a less effective or failing suppression of protein coagulation (Tjallingii, personal communication)3. In this case possibly, a different factor(s) may account for the aphids withdrawing their stylets from the sieve elements instead of initiating sap ingestion (E2). Arbitrary classes to characterise the distribution of E2 fractions showed no differences between Tugela and TugelaDn (Tables 4.4 and 4.5), despite the fact that the mean duration of E2 fractions and the sum of E2 fractions was significantly higher on the susceptible genotype.

TABLE 4.2 Comparison of four arbitrary classes of E1 fractions for *D. noxia* probing on previously uninfested Tugela and TugelaDn

Parameter	Tugela (Susceptible) n=19	TugelaDn (Resistant) n=21	Rank sum Tugela	Rank sum TugelaDn	Mann Whitney U	Z	p
Mean number of E1 fractions <1min.	0.21	1.67	277.5 a	542.5 b	87.5	3.033	0.0026
Mean number of E1 fractions >1<5 min.	2.47	2.10	408	412	181	0.501	ns
Mean number of E1 fractions >5<10 min.	1.79	0.90	452	368	137	1.693	ns
Mean number E1 fractions > 10 min.	1.42	2.48	317.5	502.5	127.5	1.950	ns

No difference was noted between genotypes for the number of probes made after sustained phloem ingestion was reached (Tugela = 3.21; Tugela Dn = 1.05; Mann-Whitney U-value 137.7, Z = 1.670, ns). There was also no difference in the % aphids that reached sustained phloem feeding (Tugela = 100%; TugelaDn = 85.7%; Chi^2 = 0.232, Tabled value = 14.067). There was a significant difference in *D. noxia* probing with respect to the parameters F and G with a higher number of F and G periods on TugelaDn (Figure 4.5) as well as a significantly larger sum of F and G periods on the resistant line (Figure 4.6). No difference was noted for the number of E1 periods followed by and F or G period between genotypes.

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TABLE 4.3 Row x Column Chi^2 test of the total observed frequencies of the four classes of E1 fractions of *D. noxia* probing on previously uninfestedTugela and TugelaDn

Parameter	Tugela	TugelaDn	Total
Number of E1 fractions <1minute	4	35	39
Number of E1 fractions >1<5 minutes	47	44	91
Number of E1 fractions >5<10 minutes	34	19	53
Number E1 fractions > 10 minutes	27	52	79
Total	112	150	262
Chi^2=32.06, df=3, p=0.05, Tabled Chi^2=7.815			

TABLE 4.4 Comparison of four arbitrary classes of E2 fractions for *D. noxia* probing on previously uninfested Tugela and TugelaDn

Parameter	Tugela (Susceptible) n=19	TugelaDn (Resistant) n=21	Rank sum Tugela	Rank sum TugelaDn	Mann Whitney U	Z	p
Mean number of E2 fractions <10min.	2.79	3.62	355.5	464.5	165.5	0.921	ns
Mean number of E2 fractions >10<20 min.	0.84	0.48	436.5	383.5	152.5	1.273	ns
Mean number of E2 fractions >20<60 min.	0.84	0.62	403.0	417.0	186.0	0.366	ns
Mean number E2 fractions > 60 min.	0.89	0.81	400.5	419.5	188.5	0.298	ns

TABLE 4.5 Row x Column Chi^2 test of the total observed frequencies of the four classes of E2 fractions of *D. noxia* probing on previously uninfested Tugela and TugelaDn

Parameter	Tugela	TugelaDn	Total
Number of E2 fractions < 10 minutes	53	76	129
Number of E2 fractions >10<20 minutes	16	10	26
Number of E2 fractions >20<60 minutes	16	13	29
Number of E2 fractions > 60 minutes	17	17	34
Total	102	116	218
Chi^2=4.917, df=3, p=0.05, Tabled Chi^2=7.815			

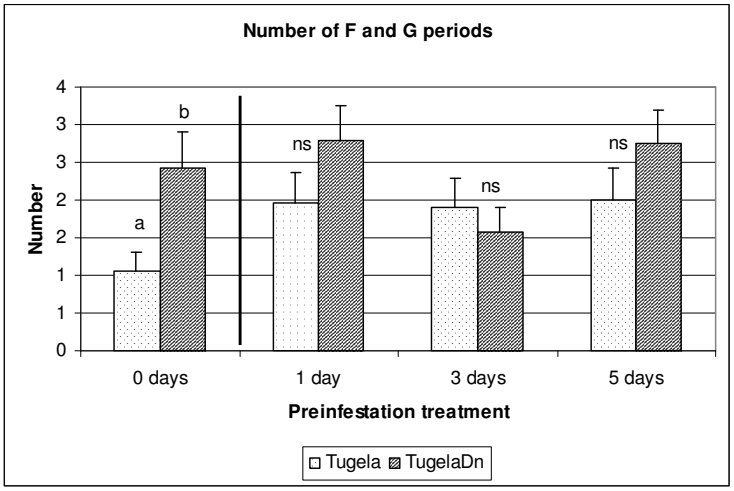

FIGURE 4.5 Number of F and G periods for *D. noxia* probing on susceptible Tugela and resistant TugelaDn, preinfested for one, three and five days (induced resistance). (^{abc} means within a preinfestation treatment without letters in common differ significantly, Mann Whitney U-Test ($p<0.05$)).

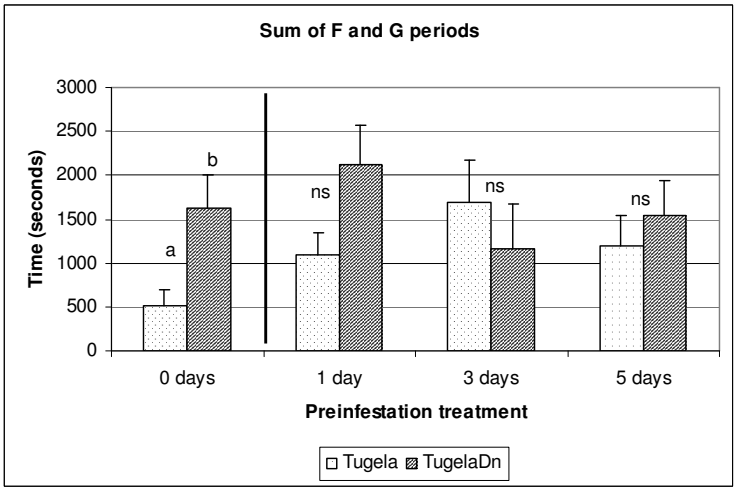

FIGURE 4.6 Sum of F and G periods for *D. noxia* probing on susceptible Tugela and resistant TugelaDn, preinfested for one, three and five days (induced resistance). (^{abc} means within a preinfestation treatment without letters in common differ significantly, Mann Whitney U-Test ($p<0.05$)).

Induced effects : Tugela vs TugelaDn [Tug-1 vs TugDn-1; Tug-3 vs TugDn-3; Tug-5 vs TugDn-5]

No differences occurred between *D. noxia* on Tugela and TugelaDn for the duration of the first probe, the sum of probing, sum of pathway and number of probes at any of the preinfestation times. One exception was the number of path periods on plants preinfested for five days (Figure 4.7) indicating more but shorter probes by *D. noxia* on the resistant plants. The time to the first phloem phase in the experiment, as well as within a probe did not differ between genotypes at any of the preinfestation treatments, neither did the number of short probes (<3 min) recorded before the first sustained phloem ingestion. This confirms indications from previously uninfested treatments that no epidermal, mesophyll or vascular parenchyma factors are involved in the resistance in TugelaDn.

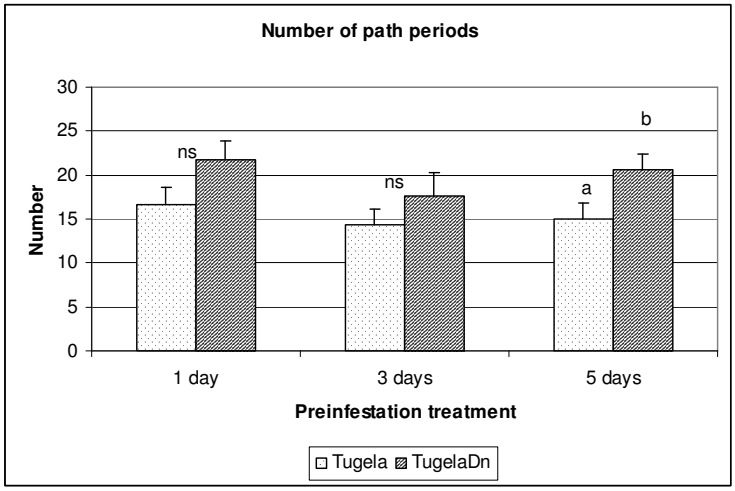

FIGURE 4.7 Number of path periods for *D. noxia* probing on susceptible Tugela and resistant TugelaDn, preinfested for one, three and five days (induced resistance). (^{abc} means within a preinfestation treatment without letters in common differ significantly, Mann Whitney U-Test ($p<0.05$)).

Although *D. noxia* had no difficulty in accessing the phloem on previously infested TugelaDn, the aphids spent less time in sieve elements (sgE1 + E12) of resistant TugelaDn than on the susceptible plants preinfested for one and for five days (Figure 4.8). The number of single E1 periods was higher on TugelaDn than on Tugela, but again, for one and five days preinfestation only (Figure 4.1). This was also true for the sum of single E1 periods (Figure 4.2) and the mean duration of the single E1 periods (Figure 4.9). As was the case with previously uninfested genotypes, *D. noxia* accessed the phloem, without switching to phloem ingestion, on the one and five day preinfested TugelaDn more often than on Tugela with the same treatments indicating that initiation of phloem ingestion (E2) was easier on the susceptible genotype.

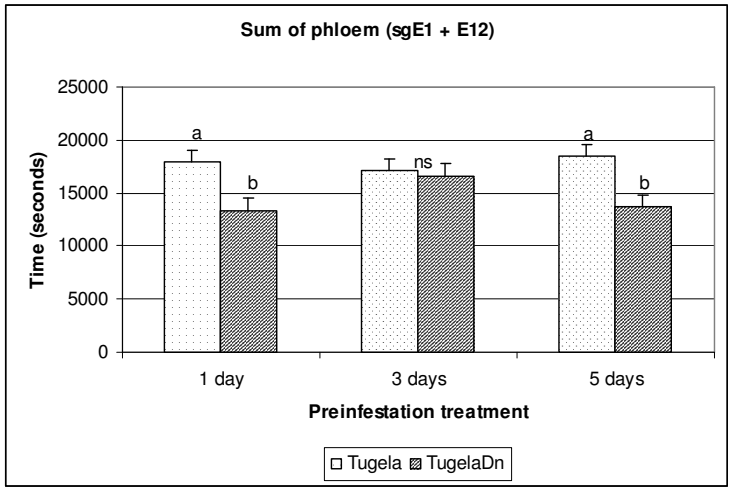

FIGURE 4.8 Sum of phloem for *D. noxia* probing on susceptible Tugela and resistant TugelaDn, preinfested for one, three and five days (induced resistance). (^{abc} means within a preinfestation treatment without letters in common differ significantly, Mann Whitney U-Test ($p<0.05$)).

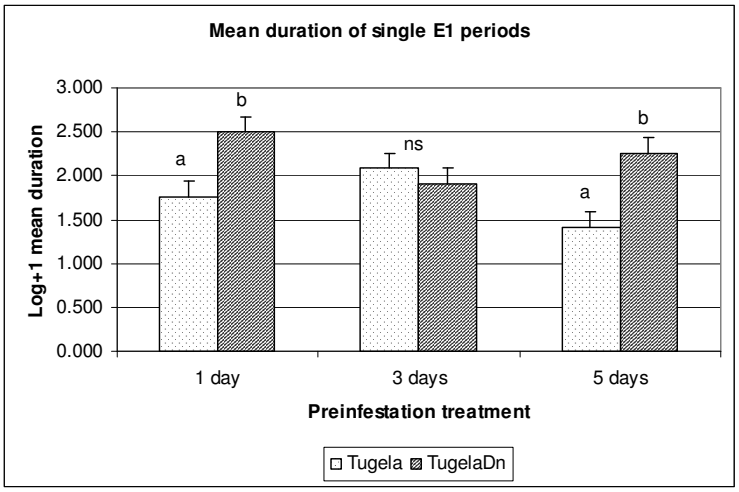

FIGURE 4.9 Mean duration of single E1 periods for *D. noxia* probing on susceptible Tugela and resistant TugelaDn, preinfested for one, three and five days (induced resistance). (^{abc} means within a preinfestation treatment without letters in common differ significantly, Mann Whitney U-Test ($p<0.05$)).

When considering E12 periods there was no difference in the number of these periods between the genotypes for any preinfestation treatment. However, the mean duration and sum of these periods was shorter on resistant TugelaDn than susceptible Tugela for the one and five day preinfestation treatments (Figures 4.10 and 4.11).

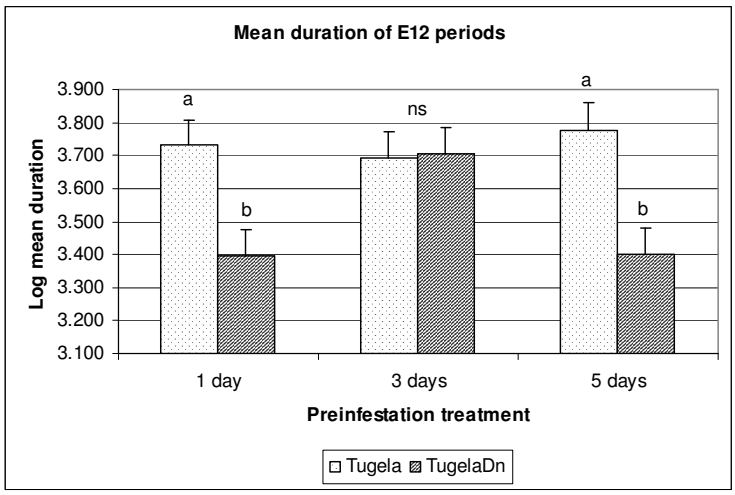

FIGURE 4.10 Mean duration of E12 periods for *D. noxia* probing on susceptible Tugela and resistant TugelaDn, preinfested for one, three and five days (induced resistance). (^{abc} means within a preinfestation treatment without letters in common differ significantly, Mann Whitney U-Test ($p<0.05$)).

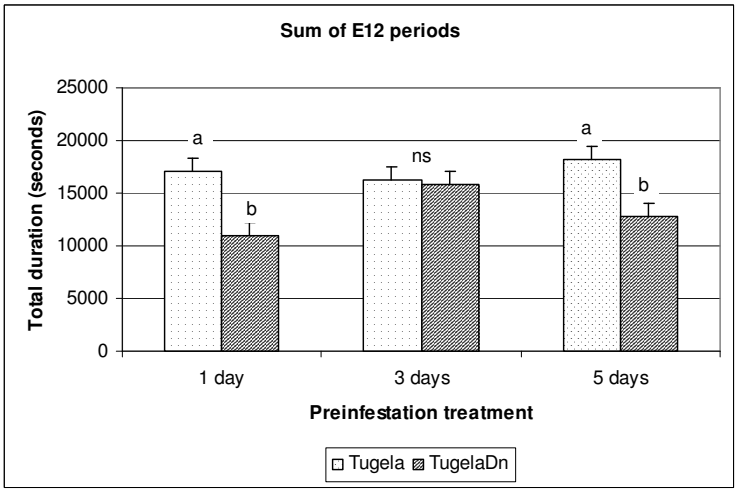

FIGURE 4.11 Sum of E12 periods for *D. noxia* probing on susceptible Tugela and resistant TugelaDn, preinfested for one, three and five days (induced resistance). (^{abc} means within a preinfestation treatment without letters in common differ significantly, Mann Whitney U-Test ($p<0.05$)).

As was the case for constitutive resistance in these genotypes, no differences were recorded for the sum, mean duration or number of E1 fractions on either of the induced genotypes at any of the prior infestation treatments. The number of E2 fractions did not differ between Tugela and TugelaDn at any of the prior infestation treatments, however the mean duration of the E2 fractions of *D. noxia* feeding on TugelaDn preinfested for one and five days was shorter than those for Tugela with the same treatments (Figure 4.3). The sum of E2 fractions was less for aphids feeding

on TugelaDn preinfested for one and five day than for those feeding on Tugela with the same treatments (Figure 4.4). This suggests that *D. noxia* were ingesting less phloem sap, which can possibly be linked to smaller body mass and slower developmental rate characteristic of antibiotic host plant resistance.

No differences were noted in the distribution of the E1 fractions, between genotypes at any of the preinfestation treatments. Again as in the case of constitutive resistance, the lack of difference between medium length E1 periods indicates that suppression of phloem protein clotting is effective in both resistant and susceptible genotypes. Differences however occur in the distribution of E2 fractions. The Row x Column Chi^2 test for the four arbitrary classes of E2 fractions, defined to characterise the distribution, shows a difference between *D. noxia* on Tugela and TugelaDn when preinfested for five days (Table 4.6). This can be explained as TugelaDn showed more E2 fractions shorter than ten minutes (Mann-Whitney U-value = 73, Tabled value = 113, p=0.05) and fewer E2 fractions longer than 60 minutes (Mann-Whitney U-value = 93, Tabled value = 113, p=0.05) than those probing on Tugela at 5 days preinfestation. Fractions shorter than ten minutes are not considered sustained phloem ingestion and a high number of these fractions indicate that the aphids were not settling into committed ingestion of nutrients.

TABLE 4.6 Row x Column Chi^2 test of the total observed frequencies of the four classes of E2 fractions of *D. noxia* on Tugela and TugelaDn previously infested for five days

Parameter	Tugela 5 days	TugelaDn 5 days	Total
Number E2 fractions < 10 minutes	29	76	105
Number E2 fractions >10<20 minutes	10	9	19
Number E2 fractions >20<60 minutes	12	13	25
Number of E2 fractions > 60 minutes	24	15	39
Total	75	113	188
Chi^2=16.188, df=3, p=0.05, Tabled Chi^2=7.815			

These results indicate that aphids are less successful in switching from salivation to ingestion on resistant plants. When they are successful in switching from phloem salivation to phloem ingestion on the resistant genotype, the salivation period on the resistant genotype is similar to that on the susceptible plants. The subsequent ingestion period is however problematic is some way, resulting in 25% more E2 fractions less than 10 minutes in duration and a smaller total ingestion of nutrients.

There were no differences in the number of F/G periods (Figure 4.5) or the sum of F/G periods (Figure 4.6) between the two genotypes at any of the preinfestation treatments. Also, there was no difference between induced Tugela and TugelaDn in the sequential parameter number of F/G

periods following an E1 period. The percentage time spent in E2 after the 1st sustained phloem feeding did not differ between the genotypes, however *D. noxia* probing on TugelaDn preinfested for one day took significantly longer to reach sustained phloem feeding than those probing on Tugela preinfested for one day (Figure 4.12). There was no difference in the number of probes made after sustained phloem ingestion on either of the genotypes at any of the preinfestation treatments and no difference in the percentage of aphids that reached sustained phloem ingestion.

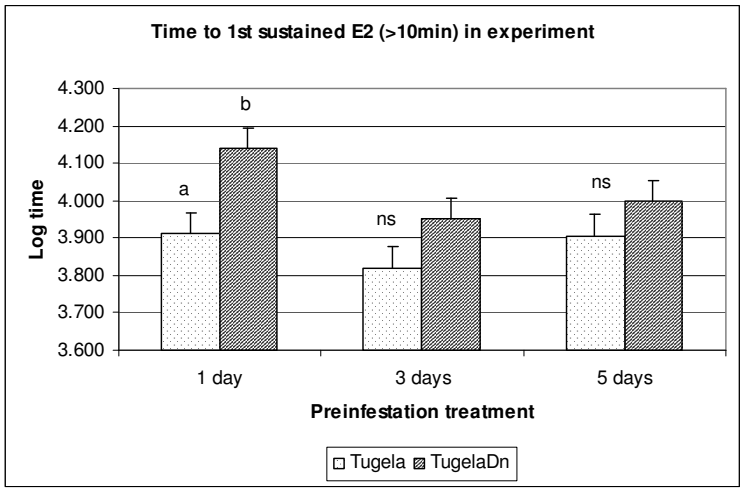

FIGURE 4.12 Time to 1st sustained E2 (>10 min) in experiment for *D. noxia* probing on susceptible Tugela and resistant TugelaDn, preinfested for one, three and five days (induced resistance). (^{abc} means within a preinfestation treatment without letters in common differ significantly, Mann Whitney U-Test ($p<0.05$)).

Clear differences are noted between the probing behaviour of *D. noxia* on previously uninfested genotypes when compared to that on previously infested genotypes. Constitutive resistance is characterised by a significantly longer time needed to attain sustained phloem ingestion on the resistant genotype, more F/G periods, a longer total duration of these waveforms, a higher number and sum of single phloem salivation periods and a shorter mean duration and sum of phloem ingestion. More phloem salivation fractions, shorter than one minute, occur on previously uninfested resistant plants in comparison to uninfested susceptible plants.

Induced resistant TugelaDn is characterised by a smaller sum of phloem activities on plants preinfested for one and five days, a shorter E12 sum and mean duration on plants preinfested for one and five days, and a shorter mean and sum of phloem ingestion on plants preinfested for one and five days. No differences are seen in the E1 fractions but significantly more E2 fractions shorter than 10 minutes and less E2 fractions longer than 60 minutes are recorded on the resistant genotype after five days preinfestation indicating a reduction in the ingestion of nutrients.

In all instances, once the phloem had been located, salivation was initiated on both genotypes but more time was spent on salivation that did not progress to ingestion (E2 periods) on the resistant line than on the susceptible one and the initiation of phloem ingestion appears easier on susceptible Tugela with a higher number of single phloem salivation periods on the resistant genotype.

Main effects : Tugela vs TugelaDn [all preinfestation treatments combined]

Due to the highly variable nature of EPG data, it is customary to use large numbers of replicates for each treatment (Van Helden and Tjallingii, 2000) and analyse data using non-parametric statistics. Despite this, some seemingly obvious differences still do not test significantly different due to the large standard deviations which are often close to or sometimes even larger than the mean itself. It may therefore be useful to pool all preinfestation treatments per genotype to obtain a larger dataset for each cultivar, which can provide an overview of the effect of the resistant genotype on the probing behaviour of *D. noxia* compared to that on the susceptible. Each preinfestation treatment accounted for a similar proportion of the total dataset per genotype and the host quality of all plants was comparable. Five day preinfested susceptible plants showed chlorotic streaks and rolled leaves but no deterioration in turgidity or tissue collapse was evident.

Overall *D. noxia* probing on resistant TugelaDn showed a shorter total probing time and a higher number of probes (Table 4.7). A higher sum of pathway was recorded on TugelaDn than on Tugela and the number of path periods was also higher on the resistant line (Table 4.7). These data imply that probing process is disrupted in such a manner that less ingestion of nutrients is possible, thus relating to the known antibiotic effect of the *Dn1* resistance gene on *D. noxia*.

TABLE 4.7 Influence of Tugela and TugelaDn on *D. noxia* probing measured by the non-sequential EPG parameters reflecting probing and pathway activities (all preinfestation treatments combined).

Parameter	Tugela (Susceptible) n=78	TugelaDn (Resistant) n=79	Rank sum Tugela	Rank sum TugelaDn	Mann Whitney U	Z	p
Sum of probing	26264	25636	6785.0a	5618.0b	2458.0	2.187	0.029
Number of probes	8	10	5564.0a	6839.0b	2483.0	2.099	0.037
Duration of 1st probe	2952	2070	5992.5	6410.5	2911.5	0.595	ns
Sum of pathway	7513	9486	5257.0a	7146.0b	2176.0	3.177	0.002
Number of path periods	16	20	5259.0a	7144.0b	2178.0	3.170	0.002

Data, reflecting an overview of the phloem parameters is shown in Table 4.8. This analysis corroborates separate analyses for constitutive and induced resistance in TugelaDn.

TABLE 4.8 The influence of Tugela and TugelaDn on *D. noxia* probing measured EPG parameters relating to phloem activities (all preinfestation treatments combined).

Parameter	Tugela (Sus.) n=78	TugelaDn (Res.) n=79	Rank sum Tugela	Rank sum TugelaDn	Mann Whitney U	Z	p
Sum of phloem	17619	14541	7201.0a	5202.0b	2042.0	3.648	0.0001
Sum of single E1 periods	637	1318	4897.0a	7506.0b	1816.0	4.441	0.0000
Number of single E1 periods	3	5	4893.0a	7510.0b	1812.0	4.455	0.0000
Mean duration of single E1 period	240	269	3739.0	5577.0	1848.0	1,923	ns
Maximum duration of single E1 period	439	743	3546.5a	5769.5b	1655.5	2.765	0.0058
Sum of E12 periods	16981	13223	7304.0	5099.0	1939.0	4.009	ns
Number of E12 periods	4	4	5845.0	6558.0	2764.0	1.113	ns
Mean duration of E12 periods	7434	4528	7028.0a	5375.0b	2215.0	3.040	0.0024
Maximum duration of E12 period	12378	9311	6992.0a	5411.0b	2251.0	2.914	0.380

A Row x Column Chi^2 test showed the observed frequencies of the four classes of E1 fractions to be different for Tugela and TugelaDn (Table 4.9). This is explained by significantly more E1 fractions longer than ten minutes on TugelaDn (Mann-Whitney U-value = 2292.5, Z = 2.768, p = 0.0058), which was not observed in the separate analyses. Longer periods of sieve element salivation in periods that do switch to phloem ingestion may indicate a problem in suppressing cell wound response or the delay/lack of a signal indicating that ingestion should be initiated. Observations of the frequencies of the four classes of E2 fractions were similar to that for induced resistance with TugelaDn showing significantly more fractions shorter than ten minutes (Mann-Whitney U-value = 2269, Z = 2.851, p = 0.0046) and significantly fewer E2 fractions longer than 60 minutes (Mann-Whitney U-value = 2341.5, Z = 2.596, p = 0.0096).

TABLE 4.9 Row x Column Chi^2 test of the observed frequencies of the four classes of E1 fractions of *D. noxia* on Tugela and TugelaDn (all preinfestation treatments combined).

Parameter	Tugela	TugelaDn	Total
Number of E1 fractions <1minute	41	69	110
Number of E1 fractions >1<5 minutes	153	157	310
Number of E1 fractions >5<10 minutes	95	81	176
Number E1 fractions > 10 minutes	110	159	269
Total	299	466	865
Chi^2=12.101, df=3, p=0.05, Tabled Chi^2=7.815			

TABLE 4.10 Row x Column Chi^2 test of the observed frequencies of the four classes of E2 fractions of *D. noxia* on Tugela and TugelaDn (all preinfestation treatments combined)

	Tugela	TugelaDn	Total
Number E2 fractions < 10 minutes	148	228	376
Number E2 fractions >10<20 minutes	48	45	93
Number E2 fractions >20<60 minutes	53	49	102
Number of E2 fractions > 60 minutes	91	68	159
Total	340	390	730
Chi^2=17.258, df=3, p=0.05, Tabled Chi^2=7.815			

Overall there was no difference in the sum of F/G periods between the two genotypes, but there were more F/G periods on TugelaDn (= 2.39) than on Tugela (= 1.73) (Mann-Whitney U-value = 245, Z = 2.233, p = 0.0264). There was no difference in the number of E periods following F/G or the number of extracellular E events. Neither number of probes shorter than three minutes before the first E1, nor the time to 1st E1 in the experiment, the time to the 1st E1 in the probe or the time to 1st sustained E2 in experiment differed(Table 4.11).

TABLE 4.11 The influence of Tugela and TugelaDn on *D. noxia* probing measured by sequential EPG parameters (all preinfestation treatments combined).

Parameter	Tugela (Sus.) n=78	TugelaDn (Res.) n=79	Rank sum Tugela	Rank sum TugelaDn	Mann Whitney U	Z	p
Number of probes <3min before E	2.141	2.203	6033.5	6369.5	2952.5	0.451	ns
Time to 1st E1 in experiment	4816	5539	6008.0	6395.0	2927.0	0.541	ns
Time to 1st E1 in probe	2151	2012	6472.0	5931.0	2771.0	1.088	ns
Time to 1st sustained E2 in experiment	8858	13299	5047.5	7355.5	1966.5	3.913	ns

Overall, *D. noxia* on resistant TugelaDn showed a significantly shorter total probing time, higher numbers of probes, longer total pathway and a higher number of path periods. The effect of resistance in TugelaDn on the probing behaviour of *D. noxia* appears to be primarily phloem related with less time spent in phloem tissue well as more periods of single sieve element salivation (sgE1), and less (passive) phloem ingestion (E2) being recorded. The mean of E12 periods was longer on Tugela (7434 seconds ≈ 124 min) than on resistant TugelaDn (4528 seconds ≈ 75 min) and can be explained by the much longer duration of the E2 fractions (Tugela ≈ 95 min; TugelaDn ≈ 47 min) as there was no difference in the mean duration of the E1 fractions. *D. noxia* on TugelaDn exhibited more E1 fractions with a duration of more than 10 minutes, possibly denoting a

different response to each genotype in terms of suppression of sieve element wound responses (Knoblauch and Van Bel, 1998) or feedback regarding the nutritional status of the sieve element. *D. noxia* showed more E2 fractions shorter than ten minutes and fewer E2 fractions longer than 60 minutes on TugelaDn than on Tugela suggesting possible dissatisfaction with the sieve element as food source.

A dendrogram illustrating the dissimilarities of the two genotypes with four pre-EPG treatments is shown in Figure 4.13. Three distinct groups are discernable with uninfested, susceptible Tugela (Tug-0) in its own group, most dissimilar to resistant TugelaDn previously infested for five days (TugDn-5), which was grouped together with resistant TugDn-1 and TugDn-0. The remaining susceptible Tugela treatments (Tug-1, Tug-3 and Tug-5) are grouped with resistant TugDn-3 in an intermediate group. It is unknown why TugelaDn, preinfested for three days, seemingly does not negatively influence *D. noxia* probing.

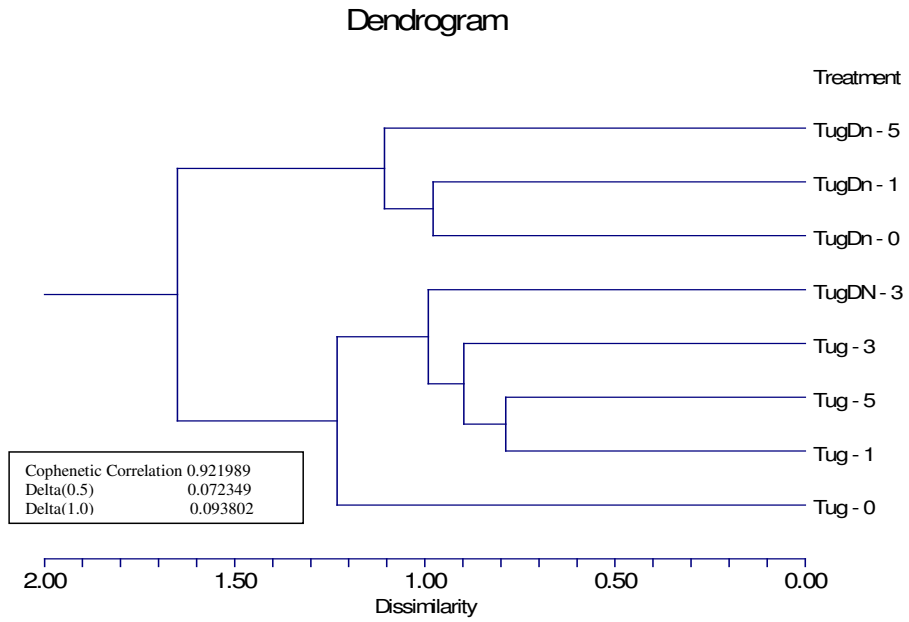

FIGURE 4.13 Cluster analyses to illustrate the relative association between probing behaviour of *D. noxia* on resistant and susceptible genotypes pre-conditioned for none, one, three and five days.

Discussion and Conclusions

In the combined analyses, as was the case with the separate analyses of both the constitutive and induced components, no indications of resistance factors were found in the epidermal and mesophyll tissues of Tugela and TugelaDn. Although Bahlmann *et al*. (2003) reported the trichome

density of TugelaDn as 28 trichomes per mm^2, almost double that of Tugela which was 16.39 tricomes per mm^2 and PI 137739, the resistance donor line, which was reported as 15.67 trichomes per mm^2 by Ni and Quisenberry (1997b) it would appear, based on the EPG data from this study, that the trichome density does not influence *D. noxia* probing on these lines. *D. noxia* needed 4816 and 5539 seconds (≈ 80 and 92 minutes) on average, from the start of the experiment to initiate salivation in the sieve elements in the susceptible and resistant genotypes respectively. The time from the start of the experiment to attaining sustained phloem feeding was however longer on TugelaDn (13299 seconds ≈ 221 min) than on Tugela (8858 seconds ≈ 147 min) but this difference was not significant. The magnitude of these data are comparable to those reported for *D. noxia* by Mayoral *et al*. (1996), who recorded a slightly longer time from the start of the experiment to the initiation of phloem salvation, of various host cereal species (ranging from 87 to 291 min). The time from the start of the experiment to attaining sustained phloem feeding ranged from 160-334 min in their study.

The effect of resistance in TugelaDn on the probing behaviour of *D. noxia* appears to be primarily phloem related with less time spent in phloem tissue and no epidermal, mesophyll or vascular parenchyma factors involved in the resistance. Although much is known about the biochemical reaction of TugelaDn to *D. noxia* probing, the effect of probing on the composition of the phloem sap has not been investigated, although this has been investigated on other resistant lines. *D. noxia* infestation of TugelaDn induced a 100kD nuclear encoded polypeptide, while the synthesis of a 56kD organelle encoded polypeptide was suppressed by *D. noxia* probing in Tugela (Van der Westhuizen and Botha, 1993). Van der Westhuizen and Pretorius (1995) reported that TugelaDn metabolically has a better ability to survive the stress condition imposed on it by *D. noxia* probing than Tugela due to a higher proline content and subsequent maintenance of chloroplast integrity and photosynthesis. An increase in phenolic content was also reported for infested TugelaDn. Accumulation of infestation related proteins of different molecular mass ranges (28-33, 22-24, 18.5-19.5 and 15.5-17 kDa) in the intercellular fluids of TugelaDn was reported by Van der Westhuizen and Pretorius (1996) as was an increase of intercellular chitinase and peroxidase activity following 48h infestation of TugelaDn (Van der Westhuizen *et al*., 1998b). However, this study shows that the pathway component of *D. noxia* probing is seemingly unaffected by the induction of these compounds. Van der Westhuizen *et al.* (1998a) reported that *D. noxia* infestation on TugelaDn induced a substantial increase of ß-1,3-glucanase activity both intra-and extracellularly within 48h of infestation and follow-up studies pinpointed the build up in the cell walls of vascular bundle cells and chloroplasts (Van der Westhuizen *et al.,* 2002) while Botha *et.al.* (1998) reported a large induction of chitinase activity in the intercellular washing fluid of infested TugelaDn. Infestation of TugelaDn with *D. noxia* induces accumulation of salicylic acid (after 48-96h of infestation) and increases peroxidase activity (after 48-120h of infestation) while catalase activity (after 24-120h of infestation) is inhibited (Mohase and Van der Westhuizen, 2002).

Clarification of the exact nature of the phloem based resistance to *D. noxia* in TugelaDn will need further research as it may be based on nutritional factors, biophysical factors or a combination of

both. The nutritional value of wheat phloem sap was studied by Telang, Sandström, Dyreson and Moran (1999) who argue that *D. noxia* shows two peculiarities that could influence its nutrition. These are the distinctive nature of the damage symptoms reported in detail by Fouché, Verhoeven, Hewitt, Walters, Kriel, and De Jager (1984) and Burd and Burton (1992), and the biosynthetic capabilities of the resident *Bruchera* endosymbionts, which, for both leucine and tryptophan, show a reduction in the number of copies of biosynthesis genes (Lai, Baumann and Moran, 1996; Thao, Baumann, Baumann, and Moran, 1998). Telang *et al.* (1999) showed that *D. noxia* feeding on susceptible Arapahoe wheat induced an increase in levels of essential amino acids while resistant Halt did not show changes in amino acid composition, resulting in a nutritionally deficiency diet for *D. noxia*. Reduced ingestion of phloem sap on resistant TugelaDn, as shown in this EPG study, is consistent with the mechanisms of antibiosis and tolerance as reported in Chapter 3 and by Wang *et al*. (2004a) who described the resistance factor in line Tugela-Dn1 to be antibiotic based on aphid biomass data.

Most EPG studies conducted with Russian wheat aphid in the past were conducted with AC systems on previously uninfested plants. The results of this study are reasonably comparable with previous studies despite the difference in germplasm tested and the different equipment used. Kindler *et al.* (1992) reported that *D. noxia* spent more time in phloem feeding on the most susceptible wheatgrass than on the wheat check; time spent on in phloem feeding on two resistant wheatgrass lines was even less than this. *D. noxia* probing on the resistant wheatgrass lines spent more time in non-probing behaviour than those on the susceptible wheatgrass and the wheat check. Webster *et al.* (1993) also recorded more non-probing behaviour and 'salivation' (i.e. stylet pathway activities) on resistant barley than on the susceptible cultivars. Significantly less phloem ingestion was observed for the resistant lines compared to the susceptible cultivars. Although the increase in non-probing behaviour on resistant hosts noted in these studies was not seen in this study, there is a similarity in that the duration of phloem ingestion (comparable to the sum of all E12 periods in this study with DC system) was found to be significantly higher on the susceptible line.

Clear differences are noted between the probing behaviour of *D. noxia* on previously uninfested genotypes when compared to that on previously infested genotypes. Constitutive resistance appears to be visible in the time taken to reach sustained phloem feeding, which is significantly longer on the uninfested, and one day preinfested treatment of the resistant line, but does not differ at three or five days preinfested treatments, more F/G periods, a longer total duration of these waveforms, a higher number and sum of single phloem salivation periods and a shorter mean duration and sum of phloem ingestion. More phloem salivation fractions, shorter than one minute, occur on previously uninfested resistant plants in comparison to uninfested susceptible plants. The reported higher trichome density on resistant TugelaDn (Bahlmann *et al*., 2003) appears to have no negative effect on *D. noxia* probing. *D. noxia* probing on induced resistant TugelaDn is characterised by a smaller sum of phloem activities on plants preinfested for one and five days, a shorter E12 sum and mean duration on plants preinfested for one and five days, and a shorter

mean and sum of phloem ingestion (E2) on plants preinfested for one and five days. *D. noxia* feeding on five day induced resistant plants showed more E2 fractions shorter than ten minutes and fewer E2 fractions longer than 60 minutes indicating a reduction in the ingestion of nutrients. Further research is required for clarification of the exact nature of the phloem-based resistance to *D. noxia* in TugelaDn. In the case of both constitutive and induced resistance initiation of phloem ingestion appears easier on susceptible Tugela with a higher number of single phloem salivation periods on the resistant genotype. Reduced ingestion of phloem sap, as shown by this EPG study, is consistent with the antibiotic nature of PI 13739 resistance. Taking both known biochemical information and EPG data into account, it would seem prudent to use plants with a five day prior infestation for future EPG studies as this would give the most comprehensive indication of the host plant resistance to *D. noxia*.

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Chapter 5

Probing behaviour of *Diuraphis noxia* on near-isogenic lines with resistance ex PI 137739 and PI 262660.

Abstract

In the summer rainfall region of South Africa the most damaging pest of cultivated wheat is the Russian wheat aphid, *Diuraphis noxia.* The DC EPG technique was used to characterise probing behaviour of this aphid pest on near-isogenic, susceptible and resistant South Africa wheat genotypes. EPG data collected for *D. noxia* on two susceptible and two resistant host genotypes reveals that the interaction between the aphid and each host genotype is unique. Distinct differences are present between the two susceptible genotypes Betta and Tugela in terms of the number of path periods and the time needed from the beginning of the experiment to attain sustained phloem ingestion. The influence of resistance in 1684/Tugela *ex* SA 1684 [PI 137739 (Dn1)] and 2199/Tugela *ex* SA 2199 [PI 262660 (Dn2)] on the probing behaviour of *D. noxia* is different. While the resistance in 1684/Tugela was reflected in a lower proportion of time spent in phloem ingestion after attaining sustained phloem feeding, the resistance in 2199/Tugela was associated with a high number of probes shorter than three minutes prior to the first phloem activity denoting some host recognition or epidermal factor involved in the resistance of this genotype which was not evident in 1684/Tugela. In addition resistance in 2199/Tugela was associated with a significantly higher percentage of time spent in phloem ingestion after the first sustained phloem feeding had been attained. The mechanisms of resistance for these genotypes, as reported in a previous study, are consistent with the data from this investigation.

Keywords: Betta, PI 137739 (*Dn1*), PI 262660 (*Dn2*), *Triticum aestivum,* Tugela

Introduction

Diuraphis noxia (Kurdjumov), Russan wheat aphid, is the most damaging pest of wheat in the summer rainfall region of South Africa and individual plant yield losses as high as 90% are possible due to this aphid (Du Toit and Walters, 1984). Resistant commercial cultivars with a yield advantage over susceptible cultivars, have replaced chemical control of the aphid estimated to amount to approximately R15 million annually (Cilliers, Tolmay and van Niekerk, 1992). Yield losses of up to R30 million per annum (Swart, 1999) have been prevented as resistant cultivars form the key component of an integrated control strategy against *D. noxia* in both commercial and small-scale production situations (Tolmay Prinsloo and Hatting, 2000).

Plant breeders are faced with the challenge of ensuring durable resistance against this pest. The identification, characterisation and exploitation of genetically different, resistant sources are critical to this end, as this knowledge will enhance and expedite the release of resistant cultivars from diverse genetic backgrounds. Du Toit (1989a) reported the resistance in PI 137739 and PI 260660 to *D. noxia* to be governed by single dominant genes, which probably differ from each other. These genes were designated *Dn1* and *Dn2* respectively and the mechanisms of resistance in the donor lines shown to be antibiosis and antixenosis (Du Toit, 1987, 1989b). Various other authors also studied these sources of *D. noxia* resistance. Smith, Schotzko, Zemetra and Souza (1992) evaluated these lines and based on percentage reduction in plant height it was concluded that both lines possessed a significant level of tolerance to *D. noxia* feeding. *D. noxia* maintained on these lines displayed reduced reproductive rates 21 days after infestation, indicating the presence of low-level antibiosis. *D. noxia* on PI 137739 were found to have a significantly lower reproduction rate than on PI 262660 and Stephens, the susceptible control, in a trial conducted by Quisenberry and Schotzko (1994). This indicated that PI 137739 showed antibiosis in contrast to PI 262660 which had higher plant growth, dry weight and moisture while expressing higher leaf chlorosis and mid-leaf rolling indicating tolerance. Studies on advanced breeding lines containing resistance *ex* PI 137739 and PI 262660 (Chapter 2) indicate antibiosis in both genotypes with a low level of tolerance in only the PI 137739 line in contrast to what was reported for the donor accession PI 262660.

Cytogenetic analysis of PI 137739 by Schroeder-Teeter, Zemetra, Schotzko, Smith and Rafi (1994) identified chromosome 7D as the location of *Dn1,* confirming an earlier report by Marais and Du Toit (1993) but in addition reported a second, associated locus that conferred a lower level of resistance than the 7D locus, on chromosome 7B. *Dn2* was also mapped to chromosome 7D (Ma, Saidi, Quick and Lapitan, 1998). Indications are that the genetic nature of resistance to *D. noxia* may be complex and affected by background factors (Souza, 1998) and once donor accessions have been utilised in breeding programmes, advanced germplasm must be carefully scrutinised to fully understand the effect of resistance on the aphid pest. This study therefore aims to investigate the probing behaviour of *D. noxia* on advanced breeding lines with resistance *ex* PI 137739 and PI 262660.

Material and Methods

Plants

D. noxia susceptible Betta (KLEIN IMPACTO) [PI 591916] and Tugela (KAVKAZ/JARAL) [PI 634771] as well as two resistant, advanced breeding lines namely SA1684/4*Tugela and SA 2199/4*Tugela containing the resistance *ex* SA 1684 [PI 137739 (Dn1)] and SA 2199 [PI 262660 (Dn2)] (Du Toit, 1987, 1988, 1989a, 1989b) respectively, were used for the study. These lines will be referred to as 1684/Tugela and 2199/Tugela throughout. They were planted daily from 24th January to 11th March 2005 in a growth chamber set at 22°C and 24h light to obtain uniform plants.

For each cultivar, four 15cm diameter plastic pots containing soil previously mixed with fertiliser were prepared and three seeds were planted per pot. Once seedlings had emerged and were approximately 10cm tall, the plants were moved to a greenhouse maintained at 16°C night : 24°C day with natural light. Each day two pots of Tugela and TugelaDn were moved to a separate cubicle in the greenhouse, maintained at the same conditions as described previously, where they were infested with approximately 15-20 *D. noxia* of mixed instars from the greenhouse colony which was maintained on the susceptible cultivar Betta.

Aphids

Aphids used in the EPG experiment were the progeny of a single, apterous *D. noxia*. This colony was maintained in a separate cage in the greenhouse chamber where the main colony was kept. Adult apterae were collected early in the morning from the colony by carefully brushing them from the leaves into a Petri dish containing a filter paper disc. Aphids were brought to the laboratory where they were attached to 20µm diameter gold wire with water soluble, conductive silver glue, under a compound microscope.

EPG set-up

Daily from 14th February 2005, four susceptible and four resistant, two-week old, aphid preconditioned plants to be used in the experiment were transferred from the greenhouse to the laboratory, where they were randomly assigned a position in the Faraday cage (Figure 3.4, Chapter 3). The preferred probing site of *D. noxia* is known to be the adaxial surface of the newest growth, in the axils of leaves or within rolled leaves (Walters *et al*., 1980). On susceptible plants, using leaf tissue close to the axil proved to be too difficult with leaves being tricky to unroll and easily breaking off. Instead, the adaxial surface of the distal third of the youngest leaf was clamped in a flat position facing upward using a hairclip covered in masking tape to prevent bruising the leaf (Figure 3.1, Chapter 3). Approximately 1 h after collection in the greenhouse the wired aphids were randomly assigned to the eight available channels and the experiment was started. A GIGA-8 (constructed by Wageningen University, The Netherlands) with eight first stage amps inside a Faraday cage was used to record EPG's . A thermograph recorded temperature throughout.

Experimental design and data treatments

All plants used in the experiment were preinfested for five days prior to use. Approximately 20 replicates (each replicate with a new aphid and plant) were collected for each genotype. Eight-hour (480 min; 28800s) EPG recordings were made and the graphs were analysed using Stylet 3.0 software developed by Tjallingii in ASYST ™ (Tjallingii, 1999). Aphids falling off the leaf or becoming detached from the gold wire were not included in the analysis. EPG parameters found to be useful in describing *D. noxia* probing in previous studies (Chapter 3 and 4) were considered. Data were prepared for statistical analysis with Microsoft Excel and analysed using either the non-parametric U-Test of Mann Whitney or Chi^2 analysis (Van Ark, 1992) with $p=0.05$.

Results and Discussion

D. noxia probing behaviour on the four genotypes was not different in terms of the sum of all probing, the number of probes and the sum of all pathway activities. The number of path periods was significantly ($p<0.05$) higher on Tugela and 1684/Tugela than on Betta, with 2199/Tugela not differing from any other genotype (Table 5.1). The duration of the first probe was significantly shorter on 2199/Tugela (4471 seconds ≈ 74 min) than on Betta (10799 seconds ≈179 min), but neither genotypes differed from Tugela or 2199/Tugela (Figure 5.1).

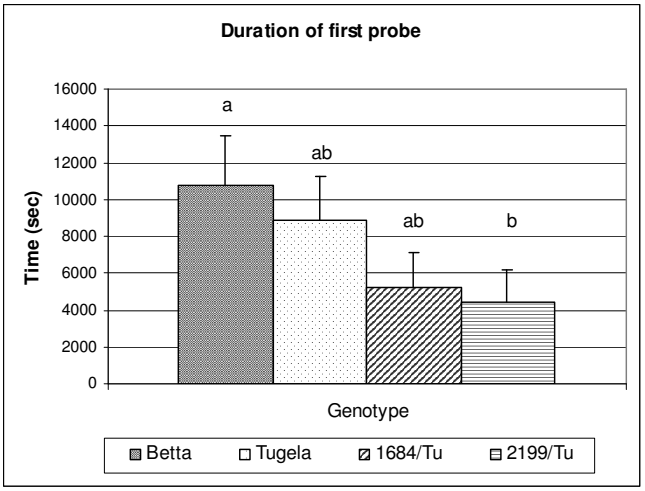

FIGURE 5.1 Duration of first probe for *D. noxia* probing on Betta, Tugela, 1684/Tugela and 2199/Tugela. (^{abc} means without letters in common differ significantly)

There were significantly more probes shorter than three minutes before the first phloem period on 2199/Tugela than on the susceptible genotypes Tugela and Betta (Figure 5.2). 1684/Tugela did not differ from either the susceptible genotypes or 2199/Tugela. When considered in context with the duration of the first probe, the high number of short probes on 2199/Tugela indicates a host recognition or epidermal factor is involved in the resistance of this genotype which is not evident in 1684/Tugela or its sister line TugelaDn (Chapter 4).

TABLE 5.1 EPG parameters recorded for *D. noxia* feeding on susceptible (Betta and Tugela) and resistant (1684/Tugela and 2199/Tugela) South African wheat genotypes. Total duration of traces = 28800 sec (8h) ([abc] parameters without letters in common differ significantly, Mann Whitney U-Test, $p<0.05$).

Parameter	Betta n=19	Tugela n=20	1684/Tugela n=20	2199/Tugela n=24
Sum of probing (sec)	26889 ±525	26723 ±536	27077 ±353	27511 ±228
Sum of pathway (sec)	7950 ±1431	10356 ±1196	10575 ±1398	7708 ±1155
Sum of phloem (sgE1 +E12) (sec)	17977 ±1611	15102 ±1498	15204 ±1658	18880 ±1327
Sum of E12 periods (sec)	17717 ±1653	14513 ±1548	14264 ±1797	18529 ±1372
Sum of E2 fractions (sec)	16551 ±1740	12712 ±1542	11152 ±1903	15806 ±1572
Sum of F/G periods (sec)	963 ±369	1266 ±402	1298 ±420	924 ±271
Number of probes	4.8 ±0.96	7.8 ±1.36	8.6 ±1.64	7.0 ±1.43
Number of path periods	9.5 ±1.51 a	15.2 ±1.72 b	16.0 ±2.21 b	12.3 ±2.15 ab
Number of single E1 periods	1.7 ±0.38	3.0 ±0.62	2.8 ±0.50	1.9 ±0.47
Number of E12 periods	2.7 ±0.30	3.7 ±0.62	3.5 ±0.71	3.2 ±0.71
Number of E1 fractions	3.4 ±0.4	5.8 ±1.1	6.1 ±1.4	5.4 ±1.2
Number of E2 fractions	2.9 ±0.31	4.5 ±0.76	4.5 ±0.91	4.4 ±0.91
Number of F/G periods *	1.3 ±0.36	1.7 ±0.44	2.3 ±0.66	1.2 ±0.33
Mean duration of single E1 periods (sec)	96 ±22	160 ±59	330 ±144	144 ±38
Maximum duration of single E1 periods (sec)	157 ±38	350 ±150	689 ±309	187 ±47
Number of E1 periods before F/G	5 ±0.13	3 ±0.08	7 ±0.13	2 ±0.06
Number of E1e periods	1 ±0.05	1 ±0.05	7 ±0.17	1 ±0.05
Time to 1st E1 in the experiment (min)	74 ±14.7	96 ±22.3	89 ±13.5	105 ±17.1
Time to 1st E1 in the probe (min)	38 ±5.0	44 ±7.0	38 ±4.4	43 ±6.6

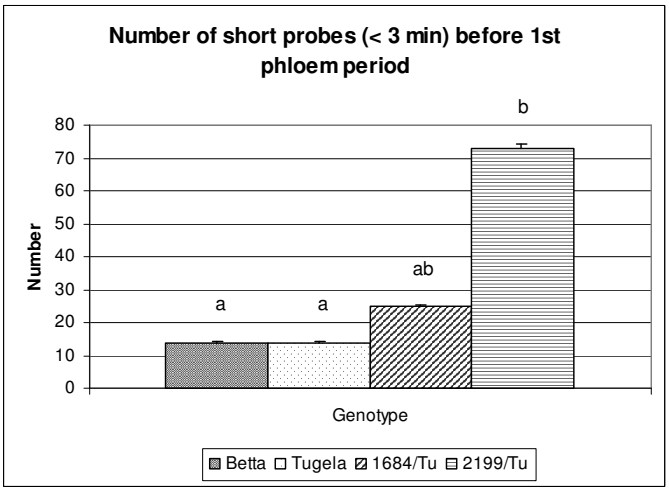

FIGURE 5.2 Number of probes shorter than 3 minutes before the first phloem period for *D. noxia* probing on Betta, Tugela, 1684/Tugela and 2199/Tugela (^{abc} means without letters in common differ significantly, Mann Whitney U-Test, $p<0.05$).

Considering the phloem activity of *D. noxia* on these genotypes, there was no difference in the total time spent in phloem activities (sum all phloem). The sum of single E1 periods was shorter on Betta than on 1684/Tugela, with Tugela and 2199/Tugela not differing from either Betta or 1684/Tugela (Figure 5.3). Although the difference between Tugela and 1684/Tugela was not significant, the trend is similar to that reported for Tugela and TugelaDn in Chapter 4. This may suggest that switching from phloem salivation to phloem ingestion is easier on the susceptible genotype. There was no difference in the number of single E1 periods between any of the genotypes (Table 5.1).

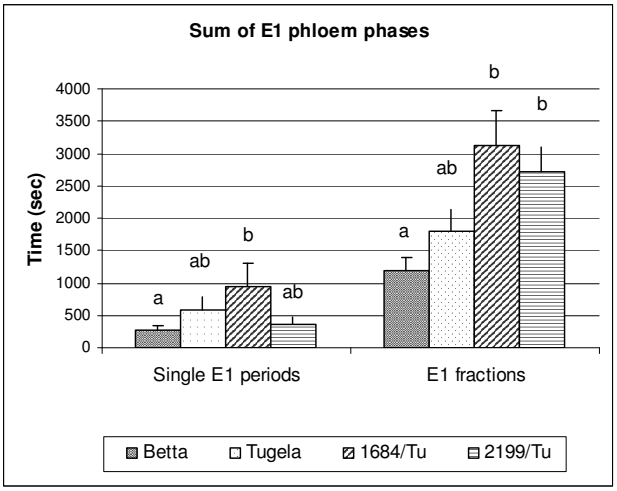

FIGURE 5.3 Sum of single E1 periods and E1 fractions for *D. noxia* probing on Betta, Tugela, 1684/Tugela and 2199/Tugela (^{abc} parameter means without letters in common differ significantly, Mann Whitney U-Test, $p<0.05$)

No differences were recorded for either the number or sum of all E12 periods (Table 5.1). Although there were no differences in the number of E1 fractions between genotypes (Table 5.1), the sum of E1 fractions was less for *D. noxia* probing on Betta than those on 1684/Tugela and 2199/Tugela, with those probing on Tugela not differing from any other genotype (Figure 5.3). A longer duration of the E1 fraction has been associated with phloem resistance and may suggest difficulties in the suppression of protein coagulation associated with wound response (Tjallingii, personal communication)4. Four arbitrary classes were defined to characterise the distribution of E1 fractions and assist in highlighting differences between genotypes. There were no differences between genotypes in E1 fractions shorter that one minute, in fractions longer than one minute but shorter than five minutes or in fractions longer than five minutes but shorter than ten minutes. There were significantly fewer E1 fractions longer than ten minutes on Betta than on 2199/Tugela (Mann-Whitney U-value = 138.0, p=0.028, Z-value = 2.201), but there were no differences between the other genotypes. The Row x Column Chi^2 test showed that the distribution of E1 fractions was significantly different between genotypes (Table 5.2) with significant differences tested between Tugela and Betta (Chi^2 = 12.135, df = 3, p =0.00833 and a Tabled Chi^2 = 11.731) as well as between Tugela and 1684/Tugela (Chi^2 = 14.616, df = 3, p =0.00833 and a Tabled Chi^2 = 11.731).

TABLE 5.2 Row x Column Chi^2 test of the observed frequencies of E1 fractions for *D. noxia* probing on Betta, Tugela, 1684/Tugela and 2199/Tugela.

	Betta	Tugela	1684/Tugela	2199/Tugela	Total
Number of E1 fractions < 1min	13	26	28	33	100
Number of E1 fractions >1<5 min	20	61	37	48	166
Number of E1 fractions >5<10 min	20	15	23	22	80
Number of E1 fractions > 10 min	11	14	32	31	88
Total	64	116	120	134	434
Chi^2 = 24.413, df=3, p=0.05, Tabled Chi^2 = 16.919					

Figure 5.4 shows the mean duration of E12 periods as well as the mean duration of the E1 and E2 fractions. The mean duration of the E12 periods for *D. noxia* probing on 2199/Tugela was significantly longer than that for *D. noxia* probing on Tugela, with Betta and 1684/Betta not differing from either of the other genotypes. The mean duration of E1 fractions was shorter for *D. noxia* probing on both Betta and Tugela than for those probing on 2199/Tugela with those probing 1684/Tugela not differing from any other genotype. No differences occurred for the mean duration of the E2 fraction. This data suggests that the nature of the phloem-based resistance to *D. noxia* in the two resistant genotypes may differ somewhat.

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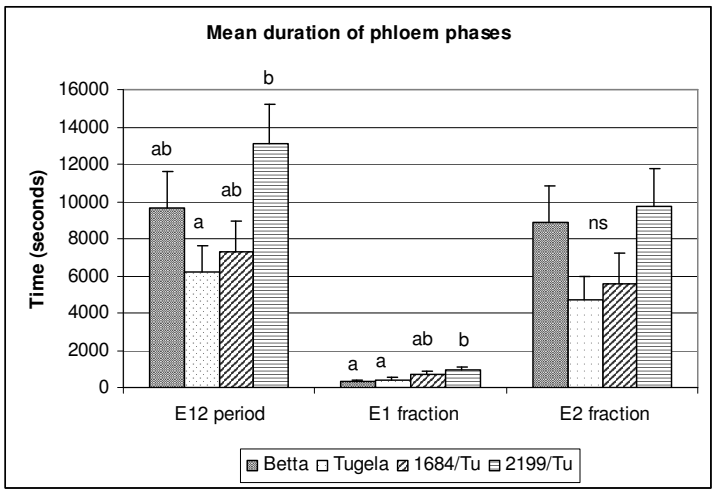

FIGURE 5.4 Mean duration of E12 periods, E1 and E2 fractions for *D. noxia* probing on Betta, Tugela, 1684/Tugela and 2199/Tugela (^{abc} means without letters in common differ significantly, Mann Whitney U-Test, $p<0.05$)

The maximum duration of the E12 period was significantly longer on 2199/Tugela than on 1684/Tugela with Betta and Tugela not differing from either the resistant genotypes (Figure 5.5). Significant differences were recorded between genotypes for the maximum duration of E1 fractions with Betta significantly shorter than both resistant genotypes, Tugela shorter than 2199/Tugela but similar to 1684/Tugela, with the resistant genotypes not differing from each other. There was no difference between the maximum duration of the E2 fraction between Betta and 2199/Tugela, which did not differ from the maximum duration of the E2 fraction on Tugela. 1684/Tugela had a significantly shorter maximum duration of the E2 fraction than all the other genotypes.

No differences were noted for the number or sum of all E2 fractions between genotypes. The Row x Column Chi^2 test however showed that the distribution of E2 fractions was significantly different between Betta and 1684/Tugela (Chi 2 = 14.882, df= 3, p= 0,05 and a Tabled Chi^2 = 11.731) and between Betta and 2199/Tugela (Chi 2 = 13.961, df= 3, p= 0,05 and a Tabled Chi^2 = 11.731) but no other differences occurred between genotypes (Table 5.3).

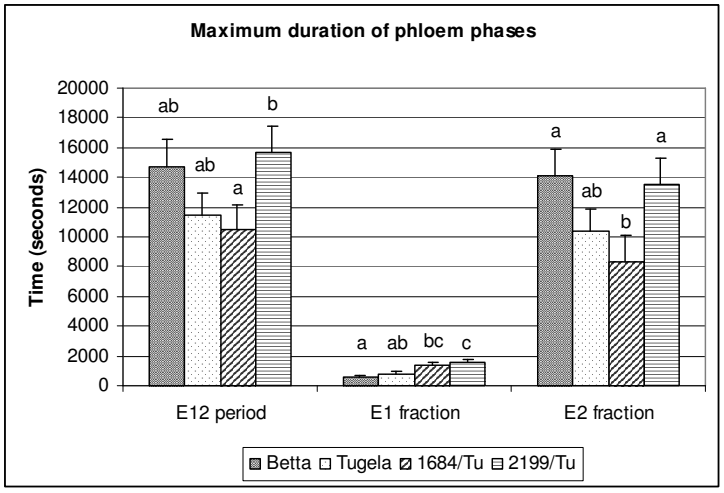

FIGURE 5.5 Maximum duration of E12 periods, E1and E2 fractions for *D. noxia* probing on Betta, Tugela, 1684/Tugela and 2199/Tugela (^{abc} means without letters in common differ significantly, Mann Whitney U-Test, $p<0.05$)

TABLE 5.3 Row x Column Chi^2 test of the observed frequencies of E2 fractions for *D. noxia* probing on Betta, Tugela, 1684/Tugela and 2199/Tugela.

	Betta	Tugela	1684/Tugela	2199/Tugela	Total
Number of E2 < 10 minutes	17	49	55	62	183
Number of E2 >10<20 minutes	8	11	5	9	33
Number of E2 >20<60 minutes	12	8	13	8	41
Number of E2 >60 minutes	19	21	15	26	81
Total	56	89	88	105	338

Chi^2 = 22.004, df=3, p=0.05, Tabled Chi^2 = 16.919

With respect to the sequential parameters there were significant differences recorded for the time to first sustained phloem ingestion, the percentage time spent in E2 after the first sustained E2 and the number of probes after the first sustained E2. *D. noxia* probing on Betta took a significantly shorter time (102 min) than those probing on Tugela (177 min) and 1684/Tugela (210 min) to reach sustained phloem ingestion (Figure 5.6). The time measured on 2199/Tugela (157 min), did not differ from any of the other genotypes.

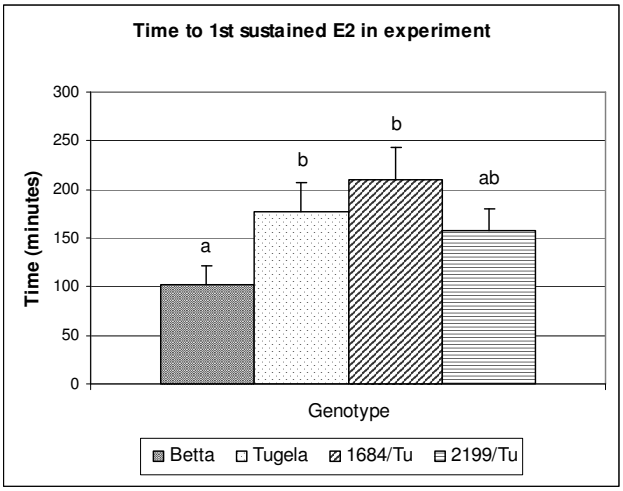

FIGURE 5.6 Time to the first sustained phloem ingestion for *D. noxia* probing on Betta, Tugela, 1684/Tugela and 2199/Tugela (^{abc} means without letters in common differ significantly, Mann Whitney U-Test, $p<0.05$)

D. noxia probing on Betta and 2199/Tugela spent significantly more time in E2 after the first sustained E2 than those aphids on 1684/Tugela, while those on Tugela did not differ from any other genotype (Figure 5.7). The highest number of probes after the first sustained E2 was recorded on Tugela, this was significantly more than on 2199/Tugela but did not differ from Betta or 1684/Tugela (Figure 5.8). No difference in D. noxia probing occurred with respect to the number of or total duration (sum of all) of F/G periods (Table 5.1).

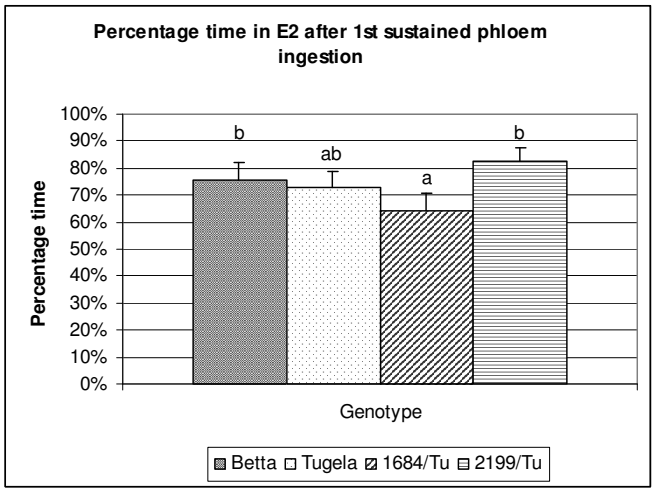

FIGURE 5.7 Percentage time in E2 after first sustained phloem ingestion for *D. noxia* probing on Betta, Tugela, 1684/Tugela and 2199/Tugela (^{abc} means without letters in common differ significantly, Mann Whitney U-Test, $p<0.05$)

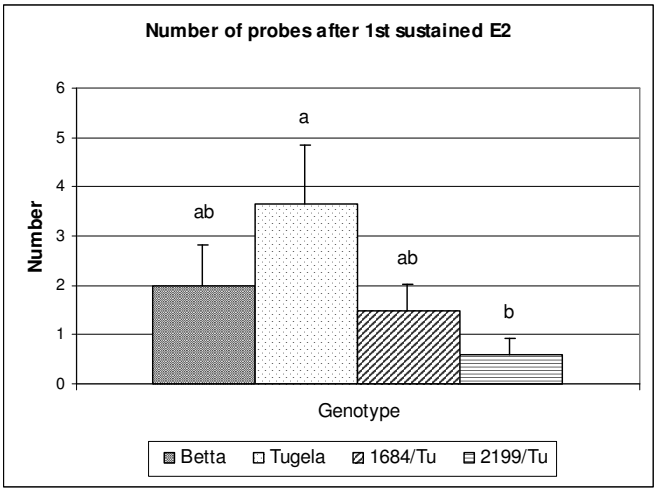

FIGURE 5.8 Number of probes after the first sustained phloem ingestion for *D. noxia* probing on Betta, Tugela, 1684/Tugela and 2199/Tugela (^{abc} means without letters in common differ significantly, Mann Whitney U-Test, $p<0.05$)

The probing behaviour of *D. noxia* on the susceptible cultivar Betta, which is known to be an excellent host of this aphid is somewhat different to that exhibited by *D. noxia* on the susceptible cultivar Tugela. Significant differences were found between the genotypes with a higher number of path periods on Tugela (15.2) than on Betta (9.5). Although not significantly so, a longer time from the start of the experiment to reach sustained phloem ingestion was recorded on Tugela (177 minutes) than on Betta (102 minutes) (Table 5.1) and significantly more E1 fractions less than one minute and more than five minutes were noted on Tugela (61) than on Betta (20). For most other parameters, the differences were not significant, however the sum of all single E1 periods on Tugela (588 seconds) was almost double of that on Betta (261 seconds) (Figure 5.3), as was the number of probes (Tugela = 7.8; Betta = 4.8; Table 5.1) and the number of single E1 periods (Tugela = 3.0; Betta = 1.7; Table 5.1). The total time spent in phloem related activities was shorter on Tugela (15102 seconds ≈ 251 min) than on Betta (17977 seconds ≈ 299 min) (Table 5.1) and aphids needed 96 and 74 minutes respectively to initiate salivation in the sieve elements from the start of the experiment on the two genotypes. This data reveals that differences exist in the probing behaviour of *D. noxia* on different susceptible host plants and may in part explain the findings of the antibiosis test (Chapter 2) in which the final mass of the *D. noxia* recovered from Tugela was less than that recovered from Betta although the number of aphids did not differ.

Using analine blue stain Botha and Matsiliza (2004) reported that *D. noxia* infested leaf tissue (wheat cv Adamtas = susceptible) was heavily callosed, with callose deposited between the plasma membrane and the cell wall, not only within the phloem tissue, but also in neighbouring vascular parenchymea cells. Deposition of wound callose was found to have disrupted phloem transport

and possibly the export of photo-assimilate from the leaves. Similar studies using Betta and Tugela may be helpful in revealing possible explanations for the findings of this EPG study.

The only significant difference for *D. noxia* probing on Tugela and 1684/Tugela was in terms of the E1 fractions where 61 E1 fractions longer than one minute but shorter than five minutes, were recorded for Tugela while only 37 were recorded for 1684/Tugela. More time was spent by *D. noxia* in single E1 periods on resistant 1684/Tugela with a shorter percentage time spent in E2 after sustained phloem ingestion has been reached on the resistant genotype, but this was not significantly different from the susceptible Tugela. This result is unexpected as EPG studies using Tugela and TugelaDn, a sister line of 1684/Tugela, showed many significant differences (Chapter 4).

Probing behaviour on susceptible Tugela was significantly different to that on the resistant genotype 2199/Tugela in terms of the mean duration of the E12 periods (Tugela = 6197 seconds; 2199/Tugela = 13108 seconds), the mean duration of the E1 fraction (Tugela = 417 seconds; 2199/Tugela = 946 seconds), the maximum duration of an E1 fraction (Tugela = 817 sec; 2199/Tugela = 1545 sec), the number of short probes before the first phloem activity (Tugela = 14; 2199/Tugela = 73) and the number of probes after the first sustained phloem ingestion (Tugela = 3.67; 2199/Tugela = 0.61).

The majority of significant differences in *D. noxia* probing occurred between host genotypes Betta and 1684/Tugela. The sum of all single E1 periods was much longer on 1684/Tugela (939 seconds) than on Betta (261 seconds), the sum of all E1 fractions on 1684/Tugela (3122 seconds) was higher than on Betta (1187 seconds), the maximum duration of the E1 fraction higher on 1684/Tugela (1331 seconds) than on Betta (637 seconds), the maximum duration of the E2 fraction shorter on 1684/Tugela (8312 seconds) than on Betta (14083 seconds), the number of path fractions higher on 1684/Tugela (16.0) than on Betta (9.5), the time from the start of the experiment to sustained phloem ingestion longer on 1684/Tugela (210 minutes) than on Betta (102 minutes) and the percentage time spent in the phloem after the first sustained phloem ingestion shorter on 1684/Tugela (64%) than on Betta (76%). Probing behaviour of *D. noxia* on Betta differed significantly to that on 2199/Tugela for the duration of the first probe (Betta = 10799 seconds; 2199/Tugela = 4471 seconds), the sum of E1 fractions (Betta = 1187 seconds; 2199/Tugela = 2723 seconds), the mean duration of the E1 fraction (Betta = 349 seconds; 2199/Tugela = 946 seconds), the maximum E1 fraction (Betta = 637 seconds; 2199/Tugela = 15456 seconds) and the number of probes shorter than 3 minutes before the first phloem activity (Betta = 14; 2199/Tugela = 73). The pertinent differences observed between Betta and the two resistant genotypes in terms of the sum and the maximum duration of the E1 fraction were however the only parameters showing significant differences common to both resistant lines eluding to a difference in the influence of the two resistant genotypes on the probing behaviour of *D. noxia.*

D. noxia probing on the two resistant genotypes was significantly different for three parameters of probing behaviour namely the percentage time spent in the phloem after sustained phloem ingestion had been achieved which was higher on 2199/Tugela (83%) than on 1684/Tugela (64%), the maximum duration of the E12 period which was higher on 2199/Tugela (15706 seconds) than on 1684/Tugela (10476 seconds) and the maximum duration of the E2 fraction which was higher on 2199/Tugela (13547 seconds) than on 1684/Tugela (8312 seconds). Mechanism of resistance studies conducted under controlled conditions showed antibiosis present in both resistant lines with that in 2199/Tugela slightly higher than that in 1684/Tugela (Chapter 2). Considering that *D. noxia* recovered from 2199/Tugela following an antibiosis test had a significantly lower mean mass than those recovered from 1684/Tugela (Chapter 2), it would appear that although more phloem ingestion is taking place on 2199/Tugela, this is not resulting in effective nutrition. This is further supported by data on the population development of *D. noxia* on these genotypes under field conditions which showed that 2199/Tugela tended to be less infested than 1684/Tugela (Chapter 2).

Conclusions

EPG data reflecting the probing behaviour of *D. noxia* on two susceptible and two resistant host genotypes reveals that the interaction between the aphid and each host genotype is unique. Distinct differences are present between the two susceptible genotypes Betta and Tugela in terms of the number of path periods and the time needed from the beginning of the experiment to attain sustained phloem ingestion and it would appear that Betta is better host for *D. noxia* than Tugela. The influence of resistance in 1684/Tugela *ex* SA 1684 [PI 137739 (Dn1)] and 2199/Tugela *ex* SA 2199 [PI 262660 (Dn2)] on the probing behaviour of *D. noxia* is different. The resistance in 1684/Tugela was generally reflected in shorter, albeit non-significant, periods of phloem activity (sgE1 +E12), a longer time required to attain sustained phloem ingestion (also not significant) and a lower proportion of time spent in phloem ingestion after attaining sustained phloem feeding. Resistance in 2199/Tugela was associated with a high number of probes shorter than three minutes prior to the first phloem activity denoting some host recognition or epidermal factor involved in the resistance of this genotype. In addition, resistance in 2199/Tugela was associated with, although not significant, fewer but longer periods of phloem salivation (E1fr), and longer periods of phloem ingestion (E2) as well as a significantly higher percentage of time spent in phloem ingestion after the first sustained phloem feeding had been attained. This would suggest that the resistance conferred by genes *Dn1* and *Dn2* differs.

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Chapter 6

Yield retention of resistant wheat cultivars, severely infested with Russian wheat aphid, *Diuraphis noxia* (Kurdjumov), in South Africa

Abstract

Russian wheat aphid, *Diuraphis noxia* (Kurdjumov), has been a serious pest of wheat in South Africa since 1978. Wheat producers observed that resistant cultivars, developed for control of this aphid, differed in resistance and questioned whether insecticide treatment would be economically justifiable. This study was undertaken to confirm and quantify the observed differences in 16 resistant cultivars under field conditions. A split-plot field trial with four replicates was planted near Bethlehem, South Africa. The yield of each aphid-infested plot was expressed as a percentage of the yield of the corresponding aphid-free plot giving percentage yield retained after infestation for each cultivar. The percentage yield retained varied in 2000 from 33.0% to 119%; in 2001 from 28.3% to 150.0% and in 2003 from 28.4% to 109.2 %. Cultivars were ranked and classified into more resistant and less resistant groups for each year and an AMMI analysis was conducted. Some cultivars compensated for *D. noxia* infestation with infested plots giving higher yield than aphid-free plots. It was shown that the level of resistance was not the same in all resistant cultivars and varied dramatically between cultivars and years when measured under severe *D. noxia* infestation.

Keywords: Damage, *Diuraphis noxia*, host plant resistance, *Triticum aestivum*, yield

Introduction

Russian wheat aphid, *Diuraphis noxia* (Kurdjumov), has been a serious pest of wheat in South Africa since its initial report in 1978. The earliest reports of *D. noxia* as an important cereal pest were from the former Soviet Union where Mokrzhetski (1914) as cited by Halbert and Stoetzel (1998) reported losses of 75% due to infestations of this aphid in 1912. Infestation of susceptible plants leads to a drastic reduction in chlorophyll content (Kruger and Hewitt, 1984; Burd and Elliot, 1996) which, when combined with the characteristic leaf rolling that occurs, causes considerable loss of effective leaf area (Walters *et al*., 1980). Yield losses as high as 60% have been reported on untreated wheat (Du Toit and Walters, 1984) in South Africa.

The use of *D. noxia* resistant cultivars in South Africa was made possible by the discovery of host plant resistance against this pest in bread wheat by Du Toit (1987; 1988; 1992). The first crosses between resistance donors and adapted South African bread wheat cultivars were made in mid-1986. The first field evaluations of back-cross progeny were undertaken in 1989 (Du Toit, 1993)

and the first resistant cultivar, Tugela-Dn, was released in 1992 (Van Niekerk, 2001). Since the early 1980's *D. noxia* has been the target of an integrated control strategy (Marasas *et al*., 1997; Tolmay, Prinsloo and Hatting, 2000) that has been actively researched and promoted to this day. This strategy has encouraged the use of a variety of natural enemies of the aphid such as parasitic wasps (Prinsloo, 1998; 2000; Prinsloo and Du Plessis, 2000), predators (Aalbersberg, Van der Westhuizen and Hewitt, 1988) and entomopathogenic fungi (Hatting *et. al*., 1999; Hatting, Poprawski and Miller, 2000; Hatting, Wraight and Miller, 2004), in support of *D. noxia* resistant cultivars, which have formed the backbone of the control programme. The use of insecticides as a curative measure has only been recommended on susceptible cultivars and in circumstances where *D. noxia* populations become very large (Tolmay *et. al.,* 2000). Observations by producers that the resistant cultivars differed in their ability to withstand *D. noxia* infestation, however led to the question of whether it would be economically justifiable to treat some of these resistant cultivars with insecticides.

Initial studies with Gamtoos-Dn (Tolmay, Van Lill and Smith, 1997), a *D. noxia* resistant cultivar developed but never released as it did not meet industry requirements, showed that the yield of resistant wheat was increased by treatment with imidacloprid seed dressing. The economic implications of insecticide treatment were however not addressed in this study. Van der Westhuizen and Lamprechts (2000), who treated the *D. noxia* resistant cultivar SST 363 with imidacloprid, reported an economically justifiable increase in yield. Subsequent studies over a five-year period with the cultivar Gariep showed that insecticide treatment was not economically justifiable, although yields were sometimes increased by insecticide application (Tolmay and Maré, 2000). These contradictory findings coupled with the increase in area planted to *D. noxia* resistant cultivars as new cultivars were released, led to a need to confirm and quantify the observed differences in resistant cultivars relative to each other. By 2000 16 cultivars with *D. noxia* resistance were available and this study is an attempt to characterise the level of resistance of these cultivars under field conditions.

Material and method

A split-plot field trial with four replicates was planted at the ARC-Small Grain Institute near Bethlehem [28°10'S, 28°18'E] for four consecutive years on 3 July 2000, 27 June 2001, 27 June 2002 and 4 July 2003. Each replicate was split into an insecticide treated and an untreated plot with 20 cultivars randomised within each plot. A modified Gaspardo precision planter with five rows and an inter-row spacing of 50cm was used and single rows were considered plots to allow for manageable total trial size. The within row spacing was ± 5cm and Round-Up® (glyphosate) was used to spray out paths between plots, leaving plots 5m in length for harvesting. The *D. noxia* susceptible cultivars Betta, Hugenoot, PAN 3211 and PAN 3377 were included as were the resistant cultivars Betta-Dn, Caledon, Elands (2000 and 2003), Gariep, Limpopo, PAN 3235, SST 124, SST 333, SST 363, SST 367, SST 399 (2001 and 2003), SST 936, SST 966, SST 972, SST

983 and Tugela-Dn. Standard fertilisation (3:2:1(25), 250kg.ha^{-1}) was applied to all plots at planting and all seed was treated prophylactically with Vitavax Plus® (carboxin/thiram) at the recommended dosage rate (300ml.100kg^{-1} seed), for the control of bunts and smuts. Gaucho® (imidacloprid) insecticide seed-dressing was applied to entries in the treated plot (200g.100kg^{-1} seed) and a Metasystox® (demeton-s-methyl) / Parathion® (parathion) mixture (500ml.ha^{-1} + 650ml.ha^{-1}) was applied as foliar spray during the season to ensure that plants remained aphid free throughout the trial. The untreated plots were artificially infested on 23 August 2000 and 13 October 2000; 18 September 2001; 4 September 2002 and 4 September 2003. Prior to infestation *D. noxia* were cultured on the susceptible cultivar Scheepers in the greenhouse. Seedlings, 25 to 30 cm in length, each infested with approximately 30 *D. noxia*, were cut and approximately twenty placed evenly per five metre row resulting in infestation of all plants in the row.

In the 2000 season observations shortly after plant emergence, revealed three plots with incomplete emergence due to false wireworm (Coleoptera: Tenebrionidae) infestation. These plots were disregarded and treated as missing values in statistical analyses. Wheat in all other plots emerged satisfactorily and in the subsequent seasons Lindastof® (gamma-BHC) was applied to control false wireworm. In the 2000 season the trial was harvested by hand and threshed. In the following seasons plots were harvested separately with a Wintersteiger plot harvester. Prior to grain yield determination each sample was individually cleaned by hand.

In each replicate, yields obtained from the untreated plot of each cultivar were expressed as a percentage of the yield of the insecticide treated control plot of the same cultivar. This will be referred to as percentage yield retained and allows cultivars to be compared to each other. Data, expressed as percentage yield retained after infestation, were subjected to analysis of variance (ANOVA) (GenStat, 2000). The multiple t-distribution test procedure of Gupta and Panchapakesan (1979) was used to rank the cultivars and classify them into two groups, a more resistant and less resistant group, with a 95% probability for the correct decision. Data for the 2000, 2001 and 2003 seasons are provided in this paper. Data from the 2002 season are not presented. These were not reliable with an extremely high co-efficient of variance, possibly due to cold damage which occurred before and during anthesis, followed by severe heat which occurred during grain filling. Data were also subjected to Additive Main effects and Multiplicative Interaction (AMMI) analyses (Gauch, 1990) and an AMMI-biplot was drawn. The AMMI model produces adjusted means that have greater predictive accuracy and are helpful in determining the reaction of cultivars in an environment (Steyn, *et. al.,* 1993).

Results and Discussion

Rainfall data for the 2000, 2001 and 2003 seasons are presented in Figure 6.1. All three seasons had higher than average rainfall with substantially more rain recorded in the pre-season (December

to June) for 2000 and within the season (July to November) in 2001. The averages for grain yield for the susceptible and resistant cultivars respectively were 2.14 and 3.08 $t.ha^{-1}$ in 2000, 1.95 and 3.65 $t.ha^{-1}$ in 2001 and 1.59 and 2.23 $t.ha^{-1}$ in 2003. Information pertaining to the date of release, date of withdrawal from commercial production (if applicable), resistance classification and *D. noxia* resistance donor accession of each cultivar is presented with the data for percentage yield retained after infestation in Table 6.1.

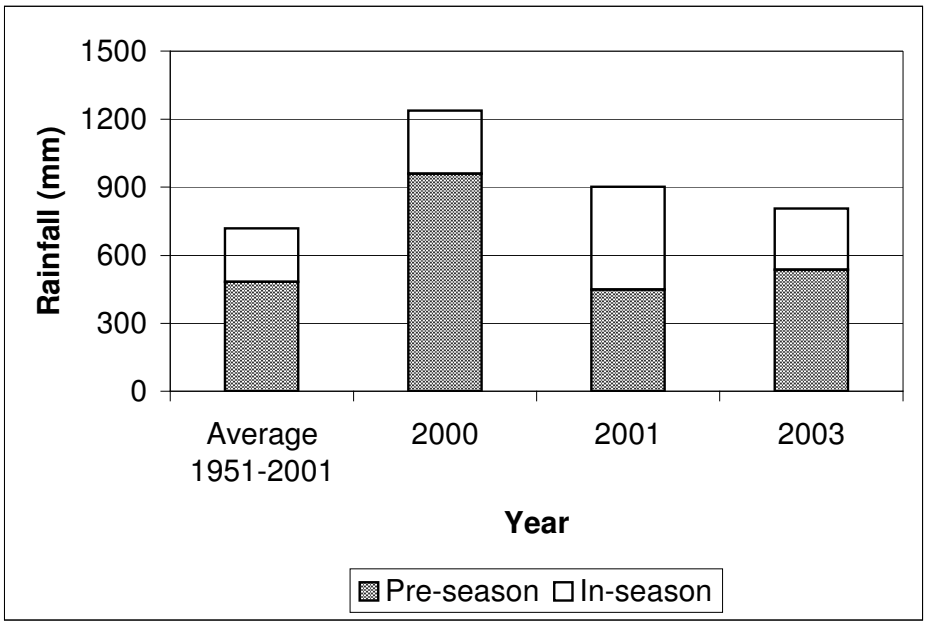

FIGURE 6.1 Long term average pre-season (December to June) and in-season (July to November) rainfall (mm) as well as the rainfall (mm) measured at Bethlehem during 2000, 2001 and 2003.

TABLE 6.1 Cultivar, date of release, date of withdrawal from commercial production, resistance classification and percentage yield retained after infestation

Cultivar	Date of release	Date of withdrawal from commercial production	Type (S=susceptible; R=Resistant)	*D. noxia* Resistance donor accession	% Yield retained		
					2000	2001	2003
Betta [1]	1970	1994	S	-	33.3	28.3	29.3
Hugenoot [2]	1989	2002	S	-	33.0	50.0	28.4
PAN 3211 [3]	1993	2000	S	-	47.6	42.0	39.9
PAN 3377 [3]	1998	-	S	-	53.0	36.3	55.2
SST 124 [2]	1987	2006	Adult Plant R	Unknown [b]	80.9	78.3	72.2
Betta-Dn [1]	1993	-	R	PI 137739	94.8	102.7	91.3
Caledon [1]	1996	-	R	PI 137739	119.0	108.3	97.8
Elands [1]	1998	-	R	PI 137739	92.3	-	109.2
Gariep [1]	1994	-	R	PI 137739	87.4	131.7	68.1
Limpopo [1]	1994	-	R	PI 137739	55.8	65.0	84.7
PAN 3235 [3]	1995	-	R	PI 262660 [a]	57.8	73.3	90.2
SST 333 [2]	1993	2006	R	PI 262660 [b]	104.9	106.7	97.4
SST 363 [2]	1996	2004	R	PI 294994 [b]	101.7	131.7	96.0
SST 367 [2]	1996	2006	R	PI 137739 [b]	87.2	148.7	82.9
SST 399 [2]	1999	-	R	PI 137739 [c]	-	133.0	106.6
SST 936 [2]	1993	-	R	PI 137739 [b]	82.7	122.0	92.8
SST 966 [2]	1996	-	R	PI 137739 [b]	67.8	94.0	77.1
SST 972 [2]	1997	2002	R	PI 294994 [b]	61.8	150.0	95.6
SST 983 [2]	1998	2006	R	PI 294994 [c]	84.7	120.0	81.7
Tugela-Dn [1]	1992	1999	R	PI 137739	93.5	100.0	67.9
Df					57	36	57
SE					3.21	15.46	13.91
LSD_T					37.44	44.33	39.38

[1] Bred by ARC-Small Grain Institute, South Africa

[2] Bred by Monsanto (Sensako Brand), South Africa

[3] Bred by PANNAR, South Africa

[a] Information provided by F. Du Toit (PANNAR) Personal communication, 1997

[b] Information provided by J. Jordaan (SENSAKO) Personal communication, 1997

[c] Information provided by D. Theunissen (Monsanto) Personal communication, 2004

The percentage yield retained under infestation varied in the 2000 season from 33.0% for susceptible Hugenoot to 119% for resistant Caledon; in the 2001 season from 28.3% for susceptible Betta to 150.0% for the resistant hybrid SST 972 and in the 2003 season from 28.4% for susceptible Hugenoot to 109.2 % for resistant Elands. In the 2000 season the variation in percentage yield retained between resistant cultivars equalled 63.2%, in the 2001 season 85.0 % and in the 2003 season 41.3%. Compensation for aphid infestation was observed in some of the cultivars where higher yields were recorded from some infested cultivars than from cultivars kept aphid free throughout the trial. This was the case for three cultivars in 2000 namely Caledon, SST 333 and SST 363; for 11 cultivars in 2001 namely Betta-Dn, Caledon, Gariep, SST 333, SST 363, SST 367, SST 399, SST 936, SST 972, SST 983 and Tugela-Dn and for two cultivars in 2003 namely Elands and SST 399. The resistant cultivars Limpopo, PAN 3235 and SST 124 always retained less than 90% yield, not showing the compensatory effect observed with the other resistant cultivars.

TABLE 6.2 Ranking and classification of cultivars into more resistant (a) and less resistant (b) groups (Gupta and Panchapakesan, 1979), with a 95% probability for the correct decision, for 2000, 2001 and 2003 seasons

	Year		
Rank	2000	2001	2003
1	Caledon a*	SST 972 a	Elands a
2	SST 333 a	SST 367 a	SST 399 a
3	SST 363 a	SST 399 a	Caledon a
4	Betta-Dn a	Gariep a	SST 333 a
5	Tugela-Dn a	SST 363 a	SST 363 a
6	Elands a	SST 936 a	SST 972 a
7	Gariep a	SST 983 a	SST 936 a
8	SST 376 a	Caledon a	Betta-Dn a
9	SST 983 a	SST 333 a	PAN 3235 a
10	SST 936 a	Betta-Dn a	Limpopo a
11	SST 124 a	Tugela-Dn a	SST 367 a
12	SST 966 b	SST 966 a	SST 983 a
13	SST 972 b	SST 124 b	SST 966 a
14	PAN 3235 b	PAN 3235 b	SST 124 a
15	Limpopo b	Limpopo b	Gariep a
16	PAN 3377 b	Hugenoot b	Tugela-Dn a
17	PAN 3211 b	PAN 3211 b	PAN 3377 b
18	Betta b	PAN 3377 b	PAN 3211 b
19	Hugenoot b	Betta b	Betta b
20	-	-	Hugenoot b

* Cultivars within columns with the same letter do not differ significantly.

The ranking and classification of cultivars (Gupta and Panchapakesan, 1979) (Table 6.2) classed the cultivars Betta-Dn, Caledon, Gariep, SST 333, SST 363, SST 367, SST 936, SST 983 and Tugela-Dn into the more resistant group in all three seasons. The cultivars Elands and SST 399 were both grouped into the more resistant group for the two seasons they were included in the experiment. The susceptible cultivars Betta, Hugenoot, PAN 3211 and PAN 3377 were included in the less resistant group for all three seasons as expected. However, SST 124, SST 966 and SST 972 were grouped into the more resistant group in two of the three seasons while Limpopo and PAN 3235 were only grouped in the more resistant group in one of the three seasons, indicating that the resistance was not expressed well in some seasons. This grouping compared well with the AMMI bi-plot interpretation of the percentage yield retained (Figure 6.2), which also placed the cultivars Betta, Hugenoot, PAN 3211 and PAN 3377 in a distinct group. The remaining cultivars, all of which were resistant, formed the second group. The cultivars SST 124, PAN 3235 and Limpopo, though clearly part of the resistant group, formed a sub group with the percentage yield retained being less than the trial mean. These cultivars were also ranked and classified in the less resistant group in certain seasons. Application of insecticides to this group of cultivars may be economically justified, but further studies should be conducted to investigate this accurately. The first IPCA was significant (F-prob = 0.0248) and accounted for 73.5% of the interaction SS.

The cultivars with IPCA scores close to zero were less sensitive to different environments and reacted in a similar way in different seasons. Cultivars with higher IPCA scores were more sensitive to environmental influence and reacted differently to *D. noxia* infestation from season to season (Figure 6.2). The AMMI selections (Table 6.3) during the 2000 and 2003 seasons were similar, while the cultivars reacted differently during the 2001 season. This may be explained by the higher than average rainfall that occurred during the growing season in 2001 while more rain occurred during the pre-season in both 2000 and 2003. SST 399 and SST 363 performed consistently well ranking high in all three years in the AMMI selection (Table 6.3), while SST 972 was more suited to the environmental conditions that prevailed in 2001 and performed well in that year. Previous studies have shown SST 363 to give an economically justifiable increase in yield when treated with insecticide (Van der Westhuizen and Lamprechts, 2000) while in this study, SST 363 retained 102%, 131% and 97% yield in 2000, 2001 and 2003 respectively which does not reflect the same trend as previously reported. Gariep retained 85%, 132% and 68% yield in the three years of this study while previous research (Tolmay and Maré, 2000) concluded that the increase in yield obtained from insecticide application seldom justified the cost thereof.

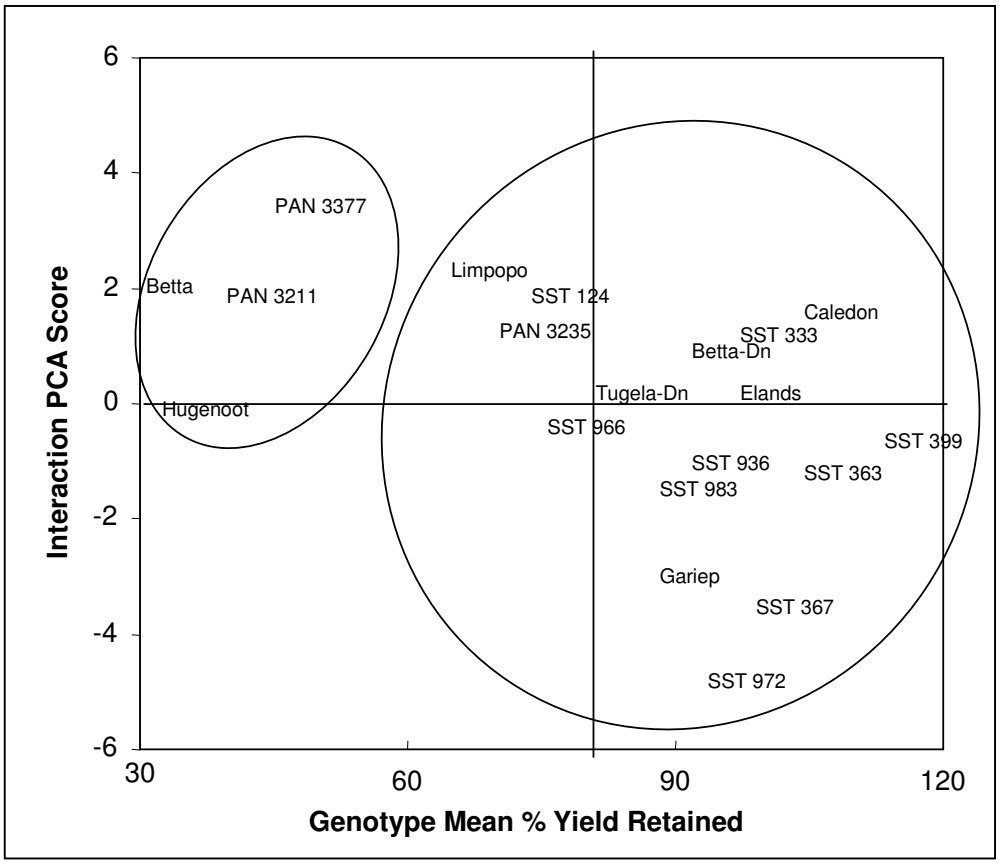

FIGURE 6.2 AMMI biplot of the first Interaction Principle Component Analysis axes for percentage yield retained after severe Russian wheat aphid infestation. Grand mean % yield retained for the trial is 82.01.

TABLE 6.3 Table of AMMI selections per year

Year	Mean	Score	AMMI selection				
2000	75.30	4.075	SST 399	Caledon	SST 333	SST 363	Elands
2001	96.00	-7.520	SST 972	SST 367	SST 399	SST 363	Gariep
2003	78.24	3.445	SST 399	Caledon	SST 333	SST 363	Elands

There was no clear indication in the AMMI-biplot (Figure 6.2) that cultivars with *D. noxia* resistance from the same donor accession (Table 6.1) reacted in a similar way. Neither was there any indication that cultivars with *D. noxia* resistance from the same donor accession show the same measure of compensation for *D. noxia* damage. Industry should however be aware that a limited number of *D. noxia* resistance donor accessions have been utilized in resistant cultivars and that new sources of resistance should be incorporated into newly released cultivars for the future as strategy to pre-empt/prevent the development of resistance breaking biotypes of *D. noxia*.

Conclusions

This study confirmed that yield loss in excess of 65% is caused by Russian wheat aphid on susceptible cultivars and emphasises the importance of including *D. noxia* resistance in new wheat cultivars for sustainable wheat production in the dryland production region of the Free State Province. The level of resistance was however not the same in all resistant cultivars and varied dramatically under severe *D. noxia* infestation. Further studies are needed to determine the economic justification and profitability of insecticide application on *D. noxia* resistant cultivars. While yield loss was demonstrated for most cultivars in this study, environmental effects appear to influence the degree of yield loss and the economic justification of insecticide application cannot be concluded from this data. In addition to the yield benefit obtained from insecticide application, which has been demonstrated in some cases, the wheat price and the cost of inputs will determine the profitability of insecticide application on *D. noxia* resistant wheat.

Since the initiation of this study other cultivars with *D. noxia* resistance have been released namely Komati, Matlabas, Nossob, PAN 3364, PAN 3120, PAN 3122, SST 322, SST 334, SST 347, SST 935 and Tarka. The percentage yield retained under severe infestation as well as the justification of insecticide application on these new cultivars should be determined. It is unlikely that cultivars will be exposed to infestation levels of the same order used in this experiment under natural field conditions. However studies to quantify *D. noxia* resistance expression, which is of utmost importance to South African wheat farmers who function on very tight profit margins in an internationally competitive market, will be of considerable benefit.

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Chapter 7
General discussion and conclusions

The Russian wheat aphid, *Diuraphis noxia* is the most economically important pest of wheat in South Africa, causing extensive damage annually (Du Toit, 1992). The use of *D. noxia* resistant cultivars may reduce the impact of this pest on cereal production, at the same time reducing environmental risks and minimizing control costs (Tolmay, Prinsloo and Hatting, 2000). Near-isogenic advanced (BC_3) breeding lines 1684/Tugela and 2199/Tugela containing *D. noxia* resistance ex SA1684 [PI 137739 (*Dn1*)] and SA 2199 [PI 262660 (*Dn2*)] (Du Toit, 1989a) were used to characterise the effect of host plant resistance on *D. noxia* in terms of the functional categories antibiosis, antixenosis and tolerance. The population development of *D. noxia* under field conditions in the Eastern Free State and the probing behaviour of *D. noxia* were also studied on these genotypes using the susceptible cultivars Betta and Tugela for comparison. A sister line of 1684/Tugela, the commercial cultivar TugelaDn, was compared to its susceptible, near-isogenic parent-line in an EPG experiment to characterise the constitutive and induced components of the resistance to *D. noxia.*

Mechanism of resistance studies conducted under controlled conditions showed antibiosis present in both advanced breeding lines, 1684/Tugela and 2199/Tugela. This correlated well with the lower *D. noxia* numbers that were recorded on the resistant lines under field conditions and is consistent with reports of antibiosis present in the original sources of resistance, PI 137739 and PI 260660, which were tested by Du Toit (1987, 1989b), Smith, Schotzko, Zemetra and Souza (1992) as well as Quisenberry and Schotzko (1994). Significantly more *D. noxia* were recovered from 2199/Tugela in the antixenosis experiment than from any of the other test entries indicating a lack of antixenosis in this line. However, this was not reflected in higher *D. noxia* infestation of this genotype under field conditions. Although not significantly so, the least *D. noxia* were recovered from 1684/Tugela in the antixenosis experiment, possibly an indication of weak antixenosis in this line, which may in part account for the lower populations of *D. noxia* recorded under field conditions. In terms of tolerance, data from this study on advanced lines, revealed only moderate to low levels of tolerance to *D. noxia* infestation in contrast to reports by Smith *et al.* (1992), and Quisenberry and Schotzko (1994) who indicated significant levels of tolerance particularly in the donor accession PI 262660. 1684/Tugela was able to retain the same leaf area and plant dry mass as 2199/Tugela under a much higher aphid infestation level and both lines were significantly more tolerant than the susceptible control Betta, but did not differ from the other susceptible control, Tugela.

In the field trial, the 1993 season was characterized by high yields and very low *D. noxia* numbers in contrast to the 1994 season where severe *D. noxia* infestations and lower yields occurred. The percentage of infested tillers was higher on the susceptible cultivars Betta and Tugela than on the resistant lines, SA1684/Tugela and SA2199/Tugela, during both seasons. During both seasons the percentage of tillers infested on the resistant lines was much lower than on the susceptible

controls, reaching maximums of 12% and 50% during 1993 and 1994 respectively. The number of *D. noxia* per tiller was also lower on the resistant lines during both seasons varying from a mean maximum of three aphids per infested tiller on the resistant lines to a mean maximum of 50 aphids per infested tiller on the susceptible lines. A comparison between this data and that of Aalbersberg (1987) who studied the population build-up of *D. noxia* in the susceptible cultivar Betta from 1983-1985, showed that the initial rapid increase of *D. noxia* which began in mid-September in the early eighties only began in the beginning of October in the early nineties. Peak aphid numbers were reached towards the end of October in the years studied by Aalbersberg (1987) while they were only reached in early to mid-November in 1993 and 1994. During 1993 and 1994 only a limited area was planted to *D. noxia* resistant cultivars. Attempts at repeating this experiment in 2004 failed due to extremely low population levels of *D. noxia,* which precluded the collection of sufficient data to allow statistical analysis and drought conditions which resulted in termination of the trial. The very low field populations in 2004 may possibly be explained by the widespread use of resistant cultivars for *D. noxia* control, which has been estimated at approximately 70 % of the area planted to wheat in the Free State (Tolmay, 2001). This is thought to significantly hamper population build-up both during and between seasons, as most volunteer wheat, a significant between season host of the aphid (Kriel, Hewitt, De Jager, Walters, Fouchè and Van der Westhuizen, 1984), is resistant and *D. noxia* resistant wheat has a lower percentage of tillers infested and fewer *D. noxia* per infested tiller. Smith (1989) states that more than one mechanism can be present in a given line and the resistance reaction depends to some extent, on the genetic background of the line. This appears to be the case with these genotypes where low to moderate levels (non-significant) levels of antibiosis, antixenosis or tolerance measured in controlled environment studies of advanced lines manifest as biologically significant levels of field resistance, giving acceptable control of the target pest. The environment also plays a very important role in the expression of resistance with host plant resistance to *D. noxia* most valuable to wheat producers in environmental conditions that favour pest population development.

EPG data reflecting the probing behaviour of *D. noxia* on the same two susceptible and two resistant host genotypes reveals distinct differences are present between the susceptible genotypes Betta and Tugela in terms of the number of path periods and the time needed from the beginning of the experiment to attain sustained phloem ingestion. It would appear that Betta is better host for *D. noxia* than Tugela as probing is more efficient on this line. Betta also showed more damage in all the mechanism tests and is severely damaged under field conditions. The influence of resistance in 1684/Tugela *ex* SA 1684 [PI 137739 (Dn1)] and 2199/Tugela *ex* SA 2199 [PI 262660 (Dn2)] on the probing behaviour of *D. noxia* is different indicating that they are indeed two separate genes, with different modes of action. While the resistance in 1684/Tugela was generally reflected in shorter, albeit non-significant, periods of phloem activity (sgE1 + E12), a longer time required to attain sustained phloem ingestion (also not significant) and a lower proportion of time spent in phloem ingestion after attaining sustained phloem feeding, the resistance in 2199/Tugela was associated with a high number of probes shorter than three minutes prior to the first phloem activity denoting some host recognition or epidermal factor involved in the

resistance of this genotype. In addition resistance in 2199/Tugela was associated with fewer but longer periods of phloem salivation (E1fr)and longer periods of phloem ingestion (E2) athough these differences were not significant. Further, a significantly higher percentage of time was spent in phloem ingestion after the first sustained phloem feeding had been attained. The observation that 1684/Tugela exerts a stronger negative influence on *D. noxia* probing than 2199/Tugela is supportive of the study by Ni and Quisenberry (1997) which reported the same trend for the two donor accessions PI 137739 and PI 262660.

The first *D. noxia* resistant cultivar to be released in South Africa was TugelaDn (Van Niekerk, 2001). This cultivar was withdrawn from commercial production in 1999, but still plays a valuable role in research by virtue of the fact that it is the most studied of all *D. noxia* resistant genotypes. Constitutive resistance in TugelaDn influences the time taken by *D. noxia* to reach sustained phloem feeding, which is significantly longer on the uninfested, and one day preinfested treatment of the resistant line, but does not differ at three or five days preinfested treatments. The reported higher trichome density on resistant TugelaDn (Bahlmann, Govender and Botha, 2003) appears to have no negative effect on *D. noxia* probing as there is no evidence of surface or epidermal factors influencing probing behavior. More periods of mechanical stylet work (F) and xylem ingestion (G) waveforms occur and the total duration of these waveforms is longer. Time spent in these waveforms does not contribute to ingestion of high quality nutrients and this may well have negative implications in terms of aphid growth and body mass. The mean duration and sum of phloem ingestion is shorter on previously infested resistant plants than on previously uninfested susceptible plants. More phloem salivation fractions, shorter than one minute, occur on previously uninfested resistant plants in comparison to uninfested susceptible plants. *D. noxia* probing on induced resistant TugelaDn is characterised by a smaller sum of phloem activities on plants preinfested for one and five days, a shorter E12 sum and mean duration on plants preinfested for one and five days, and a shorter mean and sum of phloem ingestion (E2) on plants preinfested for one and five days. *D. noxia* feeding on five day induced resistant plants showed more E2 fractions shorter than ten minutes and fewer E2 fractions longer than 60 minutes indicating a reduction in the ingestion of nutrients. In the case of both constitutive and induced resistance initiation of phloem ingestion appears easier on susceptible Tugela with a higher number of single phloem salivation periods on the resistant genotype. Reduced ingestion of phloem sap by *D. noxia*, on TugelaDn could account for the ability of this genotype to maintain yield despite high aphid infestation levels as found in field trials conducted over three years to quantify yield loss in resistant cultivars.

The DC EPG system gives a clear insight into the probing activities that take place within the sieve elements and highlights a number of disturbances that occur in terms of aphid probing behaviour on resistant TugelaDn. Biochemical studies on susceptible Tugela and the near-isogenic resistant cultivar Tugela-DN, by Van Der Westhuizen and Pretorius (1995) concluded that changes occurred in the chlorophyll, protein, free amino acid, proline levels and respiration rate in response to *D. noxia* infestation. Their study indicated that a stress condition is induced in both susceptible and resistant wheat plants by *D. noxia* feeding and they postulated that the unique changes in resistant

wheat, especially the marked increase in the total free proline content, seemed to contribute to the plants improved ability to cope with *D. noxia* infestation and survive. Proline is known to play a protective role for membrane systems under stress. Membranes in resistant plants remain intact and photosynthesis can proceed relatively normally as opposed to susceptible plants where the chloroplasts are damaged (Fouché, Verhoeven, Hewitt, Walters, Kriel and De Jager,1984). Although an increase in the total phenolic content in infested resistant plants may contribute a possible deterrent effect against *D. noxia,* none of the other observed biochemical changes in resistant wheat could be regarded as detrimental to *D. noxia per se*. Overall, in terms of chemical factors, there is often no inherent difference between the chemistry of constitutive and induced defences with the accumulation / up-regulation pre-existing compounds being induced by herbivore damage. In most cases of defence responses induced by insect herbivory, for both the wounding and pathogenesis pathways, saliva plays an important role in the elicitation of plant defence (Felton and Eichenseer, 2000). Increased levels of salivation of *D. noxia* probing on resistant genotypes have been reported in this study. This may possibly contribute to the stronger expression of resistance in genotypes infested for five days when compared to those infested for less which was also observed in these experiments.

Further research is required for clarification of the exact nature of the phloem-based resistance to *D. noxia* in TugelaDn. It is known that many protein-based defences in resistant plants have an anti-nutritive effect on herbivores, destroying or preventing the assimilation of nutrients by the insect thereby slowing the growth and development of the herbivore (Constabel, 2000). Studies by Lai, Baumann and Moran (1996) and Thao, Baumann, Baumann and Moran (1998) show a reduced number of copies of the biosynthesis genes for leucine and tryptophan in the *Bruchera* endosymbionts of *D. noxia*. This indicated that *D. noxia* obtain essential amino acids they require for growth and development from the phloem sap they ingest. Telang, Sandström, Dyerson and Moran (1999) confirmed that *D. noxia* feeding increased levels of essential amino acids in susceptible Arapahoe wheat, while no change was recorded in the essential amino acid composition of resistant Halt following *D. noxia* feeding. A similar case may hold true for TugelaDn and could provide a plausible explanation for longer periods of salivation and shorter periods of ingestion as revealed in this study, however the appropriate study will have to be conducted to confirm or refute this.

Another aspect that requires further investigation is that of the hydroxamic acid content of the resistant and susceptible lines used in this study. Nicol, Copaja, Wratten and Niemeyer (1992) screened worldwide wheat cultivars for hydroxamic acid levels finding that Betta had a DIMBOA (the main hydroxamic acid in wheat) level of 1.29 $mmol.kg^{-1}$ fresh weight while that of Tugela was 2.00 $mmol.kg^{-1}$ and that of the resistant donor line SA 2199 was 2.15 $mmol.kg^{-1}$. These concentrations are considered medium where a level of above 3.4 would be considered high (Givovich and Niemeyer 1996). DIMBOA has been found to exert both toxic and antifeedant effects on other grain aphids (Nicol *et al*., 1992) while Givovich and Niemeyer (1996) reported that higher DIMBOA levels in wheat seedlings led to lower mean relative growth rates of *D. noxia*.

Determination of the levels of DIMBOA in the advanced lines used in this study will add value to the already available data. It is however unlikely that the levels of these hydroxamic acids are very high in the advanced lines used in this study, given the low to medium levels present in the parent genotypes.

Electro penetration graph studies confirmed that the DC EPG technique can highlight differences in probing behaviour of *D. noxia* on various resistant genotypes, however, from this study it is apparent that eight hour recordings should be made and plants that have been preinfested for five days should be used in these experiments. This method is not suitable for use as a screening procedure within a breeding programme given the highly variable nature of the data and the large number of replicates needed to draw accurate conclusions. Valuable information can however be collected leading to a better understanding of the underlying basis of host plant resistance to *D. noxia* which may allow better manipulation of resistance in the future.

D. noxia resistant cultivars have been shown to be very effective at controlling this pest in producers' fields. With low levels of aphid infestation occurring from as early as August in most seasons, constitutive resistance may be effective for a short period, rapidly being followed up by induced resistance which probably plays a more important role in influencing population development throughout the season. In addition to limiting *D. noxia* numbers, the maintenance of chloroplast and membrane integrity (Van Der Westhuizen and Pretorius, 1995) due to up regulation of proline gives these cultivars the ability to continue with photosynthesis and maintain water balance, two critical aspects which have been found responsible for yield loss in susceptible genotypes (Burd and Burton, 1992).

Yield data for five susceptible and 15 resistant cultivars was analysed using an AMMI-biplot. Cultivars known to contain *D. noxia* resistance *ex* PI 137739 are BettaDn, Caledon, Elands, Limpopo, SST 367, SST 399, SST 936, SST 966 and TugelaDn, while PAN 3235 and SST 333 contain resistance *ex* donor PI 262660. Cultivars with *D. noxia* resistance from the same donor accession did not react in a similar way, neither was there any indication that cultivars with *D. noxia* resistance from the same donor accession show the same measure of compensation for *D. noxia* damage. Based on the percentage yield retained by each genotype the susceptible cultivars Betta, Hugenoot, PAN 3211 and PAN 3377 formed a distinct group in the AMMI bi-plot. The remaining cultivars, all of which were resistant, made up a second group, but the cultivars SST 124, PAN 3235 and Limpopo, though clearly part of the resistant group, formed a sub group with the percentage yield retained being less than the trial mean. Cultivars were also ranked and classified into more and less resistant groups. The ranking and classification of cultivars classed the cultivars Betta-Dn, Caledon, Gariep, SST 333, SST 363, SST 367, SST 936, SST 983 and Tugela-Dn into the more resistant group in all three seasons. The cultivars Elands and SST 399 were both grouped into the more resistant group for the two seasons they were included in the experiment. The susceptible cultivars Betta, Hugenoot, PAN 3211 and PAN 3377 were included in the less resistant group for all three seasons as expected. However, SST 124, SST 966 and SST

972 were grouped into the more resistant group in two of the three seasons while Limpopo and PAN 3235 were only grouped in the more resistant group in one of the three seasons, indicating that the resistance was not expressed well in some seasons. In the 2000 season the variation in percentage yield retained between resistant cultivars equalled 63.2%, in the 2001 season 85.0 % and in the 2003 season 41.3%. This is a considerable variation and confirms the important role that environmental conditions play in the expression of host plant resistance (Smith, 1989)

Some cultivars such as Elands, SST 399, SST 966 and TugelaDn were less sensitive to different environments and reacted in a similar way in different seasons. Others such as SST 367 and SST 972 were more sensitive to environmental influence and reacted differently to *D. noxia* infestation from season to season. This may be explained by varying rainfall that occurred during the different seasons. SST 399 and SST 363 performed consistently well in all three years, while SST 972 was more suited to the environmental conditions that prevailed in 2001 and performed well in that year.

The economic benefit of the use of insecticides on *D. noxia* resistant cultivars was not investigated in this study. Some research in this regard has been conducted previously with specific cultivars. Van der Westhuizen and Lamprechts (2000) showed SST 363 to give an economically justifiable increase in yield when treated with insecticide while in this study, SST 363 retained 102%, 131% and 97% yield in 2000, 2001 and 2003 respectively which does not reflect the same trend as previously reported. Gariep retained 85%, 132% and 68% yield in the three years of this study while previous research (Tolmay and Maré, 2000) concluded that the increase in yield obtained from insecticide application seldom justified the cost thereof. This study confirmed that yield loss in excess of 65% is caused by Russian wheat aphid on susceptible cultivars and emphasises the importance of including *D. noxia* resistance in new wheat cultivars for sustainable wheat production in the dryland production region of the Free State Province. While yield loss was demonstrated for most cultivars in this study, environmental effects influenced this and the economic justification of insecticide application cannot be concluded from this data. Further studies are needed to determine the economic justification and profitability of insecticide application on *D. noxia* resistant cultivars.

This study confirms that the interaction between the aphid and each host genotype is unique. The expression of host plant resistance is not only dependent on the donor accession utilised, but also the genetic background in which it is deployed. Furthermore, environmental factors can also significantly influence the performance of host plant resistance to *D. noxia*, making its successful exploitation a complex and challenging directive for plant breeders and entomologists alike.

A new, resistance breaking biotype of *D. noxia* was reported during 2005 and confirmed in greenhouse evaluations in early 2006 (Tolmay, Lindeque and Prinsloo, 2006). The resistant cultivars BettaDN, Caledon, Elands, Gariep, Komati, Limpopo, PAN 3235, PAN 3364, SST 322,

SST 334, SST 399, SST 935 and SST 966 which were marketed in the 2005 season all show significantly more damage when infested by the new biotype. Included amongst these are cultivars containing resistance *ex* PI 137739 and PI 262660. Although the cultivars mentioned are not as badly damaged as susceptible cultivars, the level of resistance is insufficient to provide protection against yield loss and it can be concluded that the resistance *ex* PI 137739 and PI 262660 is no longer effective in the field. The original population of *D. noxia,* predominant in South Africa prior the development of the new biotype, and used throughout this study, has been designated RWASA1 (Tolmay *et al.,* 2006). Large scale screening of potential sources of resistance to the new biotype(s) is currently underway.

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Chapter 8

Summary

Near-isogenic genotypes 1684/Tugela and 2199/Tugela containing *D. noxia* resistance ex SA1684 [PI 137739 (*Dn1*)] and SA 2199 [PI 262660 (*Dn2*)] were studied in terms of antibiosis, antixenosis and tolerance, population development of *D. noxia* under field conditions and the probing behaviour of *D. noxia.* The susceptible cultivars Betta and Tugela were included for comparison. A sister line of 1684/Tugela, the commercial cultivar TugelaDn, was used to characterise the constitutive and induced components of the resistance to *D. noxia.* Mechanism of resistance studies showed antibiosis present in both resistant genotypes. No antixenosis was recorded in 2199/Tugela, with only low levels present in 1684/Tugela. 1684/Tugela was able to retain the same leaf area and plant dry mass as 2199/Tugela under a much higher aphid infestation level and both lines were significantly more tolerant than Betta, but did not differ from Tugela. Betta and Tugela showed higher infestation levels, both in terms of percentage tillers infested and number of *D. noxia* per tiller, and lower yields than the resistant genotypes. The initial rapid increase of *D. noxia* populations started in the beginning of October and peak aphid numbers were in early to mid-November. EPG data reflecting the probing behaviour of *D. noxia* revealed distinct differences between the resistant cultivars and the susceptible Betta and Tugela. The influence of resistance in 1684/Tugela and 2199/Tugela on the probing behaviour of *D. noxia* is different indicating that they contain two different genes, with different modes of action. While resistance in 1684/Tugela was generally reflected by disturbances in phloem activity, the resistance in 2199/Tugela was associated with a high number of short probes denoting some host recognition or epidermal factor involved in the resistance. Constitutive resistance in TugelaDn influenced the time taken by *D. noxia* to reach sustained phloem feeding. There was no evidence of surface or epidermal factors influencing probing behaviour in this genotype. *D. noxia* probing on induced resistant TugelaDn was characterised by a reduction in phloem activities on preinfested plants. *D. noxia* feeding on five day induced resistant plants showed more E2 fractions shorter than ten minutes and fewer E2 fractions longer than 60 minutes indicating a reduction in the ingestion of nutrients. In the case of both constitutive and induced resistance, initiation of phloem ingestion appears easier on susceptible Tugela with a higher number of single phloem salivation periods on the resistant genotype. Yield data for five susceptible and 15 resistant cultivars was analysed quantifying the percentage yield retained under severe infestation. Cultivars with *D. noxia* resistance from the same donor accession did not react in a similar way, neither was there any indication that they show the same measure of compensation for *D. noxia* damage. Compensation for aphid infestation was observed in some of the cultivars where higher yields were recorded from some infested cultivars than from cultivars kept aphid free throughout the trial. In the 2000 season, the variation in percentage yield retained between resistant cultivars equalled 63.2%, in the 2001 season 85.0 % and in the 2003 season 41.3%. This study confirms that the interaction between the aphid and each host genotype is unique. The expression of host plant resistance is not only dependent on the donor accession utilised, but also the genetic background in which it is deployed.

Furthermore, environmental factors can also significantly influence the performance of host plant resistance to *D. noxia*.

Keywords: antibiosis, antixenosis**,** constitutive resistance, *Diuraphis noxia,* EPG, induced resistance, PI 137739, PI 262660, population development, probing behaviour, tolerance, *Triticum aestivum,* yield

Opsomming

Naby-isogeniese, genotipes 1684/Tugela en 2199/Tugela met *D. noxia* weerstand vanuit die bronne SA 1684 [PI 137739 (*Dn1*)] en SA 2199 [PI 262660 (*Dn2*)] is in terme van antibiose, antixenose en verdraagsaamheid ondersoek. Populasieontwikkeling van *D. noxia* is onder veldtoestande geëvalueer en voedingsgedrag van *D. noxia* bestudeer. Die vatbare cultivars Betta en Tugela is gebruik om die konstitutiewe en geïnduseerde weerstand in hierdie genotypes te beskryf. Beide 1684/Tugela en 2199/Tugela het antibiose getoon, terwyl geen antixenose in 2199/Tugela waargeneem is nie. 1684/Tugela het lae vlakke van antixenose getoon. Albei weerstandslyne is meer verdraagsaam as die vatbare kontrole Betta, maar verskil nie van Tugela nie. 1684/Tugela is instaat om dieselfde blaaroppervlakte en droëmassa te behou as 2199/Tugela, ten spyte van baie hoër besmettingsvlakke. Betta en Tugela, toon hoër besmettingsvlakke as die weerstandsgenotipes in terme van persentasie halms besmet en aantal luise per besmette halm. Die aanvanklike vinnige toename van luisgetalle het in die begin van Oktober plaasgevind en maksimum luisgetalle is vroeg tot middel November bereik. Voedingsgedrag studies het getoon dat daar beduidende verskille tussen Betta en Tugela bestaan. Die voedingsgedrag van *D. noxia* het op die twee weerstandbiedende genotipes verskil wat bevestig dat hulle twee verskillende gene bevat, elk met sy eie metode van werking. Die weerstand in 1684/Tugela is met versteurde floeëmaktiviteit gekoppel terwyl die weerstand in 2199/Tugela met ‘n epidermale of gasheer erkenningsfaktor geassosieer word. Konstitutiewe weerstand in TugelaDn beinvloed die tyd wat *D. noxia* nodig het om volgehoue floëemingestie te bereik. Daar is geen aanduidings van enige oppervlak of epidermale faktore wat die voedingsgedrag beinvloed nie. *D. noxia* voeding op geïnduseerde TugelaDn is gekenmerk deur ‘n afname in floëemaktiwiteit op plante wat vooraf besmet is. *D. noxia* op vyf dae voorafbesmette plante het meer E2 fraksies, korter as tien minute, en minder E2 fraksies langer as 60 minute getoon wat op ‘n verminderde inname van voedingstowwe dui. In die geval van beide konstitutiewe en geïnduseerde weerstand, blyk die oorskakeling van floëemspeekselafskeiding tot floëemingestie makliker te wees op die vatbare genotipe, met ‘n betekensvol hoër aantal enkel floëemspeekselafskeiding periodes (sgE1) op die weerstandbiedende cultivar. Opbrengsdata vir vyf vatbare en 15 weerstandbiedende cultivars is gebruik om die persentasie opbrengsverlies onder strawwe besmetting te bepaal. Cultivars met weerstand vanuit dieselfde bron het egter nie op ‘n soortgelyke wyse reageer nie en daar is geen aanduiding dat cultivars met dieselfde bron van weerstand soortgelyke kompensasie vir *D. noxia* skade toon nie. Kompensasie vir luisbesmetting is by sommige van die cultivars waargeneem waar hoër opbrengste op die besmette behandeling as op die luisvrye behandeling waargeneem is. Die variasie tussen die weerstandbiedende cultivars was 63.2% in die 2000 seisoen, 85% in die 2001 seisoen en 41% in die 2003 seisoen. Hierdie studie bevestig dat die interaksie tussen die

plantluis en elke genotipe spesifiek is. Die uitdrukking van gasheerplantweerstand is nie net afhanklik van die weerstandsdonor wat gebruik word nie, maar ook die genetiese agtergrond waarin dit ontplooi word. Verder speel omgewingsfaktore ‘n bepalende rol in die uitdrukking van die weerstand.