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Differential induction of apoplastic peroxidase and chitinase activities in susceptible and resistant wheat cultivars by Russian wheat aphid infestation

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Abstract The intercellular peroxidase and chitinase activities of three wheat cultivars [*Triticum aestivum* L. cvs 'Tugela DN', 'Molopo DN' (Gariep) and 'Betta DN'] containing the *Dn-1* gene for resistance to the Russian wheat aphid (RWA) *Diuraphis noxia* (Mordvilko) and the corresponding near-isogenic susceptible cultivars ('Tugela', 'Molopo' and 'Betta') were studied under conditions of infestation and non-infestation. The aim was to gain information on the mechanism of resistance. The resistance response was induced by RWA infestation. Infestation rapidly induced the activities of both enzymes selectively in resistant wheat to levels of magnitudes higher than those in susceptible wheat. The genetic background in which the *Dn-1* resistance gene is bred played a role and the level of activity corresponded to the level of resistance. Immunologic studies confirmed that the induction of enzyme activities was due to the induction of higher protein levels. These results indicate that peroxidase and chitinase may have a role in insect resistance.

Key words Russian wheat aphid resistance · Apoplast · Chitinase · Peroxidase

Introduction

The Russian wheat aphid (RWA), *Diuraphis noxia* (Mordvilko), causes serious damage to wheat and barley in many wheat-producing areas of the world (Smith et al. 1991). In South Africa it became recognized as a pest of wheat in 1978 (Walters et al. 1980) and is currently regarded as the most destructive insect pest of wheat.

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Control of this pest in South Africa is achieved mainly by spraying insecticides (Du Toit 1989a). Resistant cultivars, as an alternative to chemical control, contributed roughly 10% of the total wheat crop during 1996. The rapid development of new resistant cultivars has become a priority, also because of the possibility that new resistant RWA biotypes may evolve. Although it has been established that RWA resistance or tolerance is a combination of antibiosis and antixenosis (Du Toit 1989b) the contributing defence mechanism of the plant is still unknown. Knowledge of the defence mechanism and of the genes involved may contribute to more directed and efficient breeding of resistant wheat cultivars.

Aphids probe mainly intercellularly before the stylet penetrates the phloem (Pollard 1973). The apoplast is known to play an important role in the plant's defence mechanism as is the case during pathogenesis. Many defence-related products accumulate in the apoplast, including pathogenesis-related (PR) proteins (Bowles 1990). It was found that RWA infestation induced the accumulation of specific proteins in the intercellular fluids of resistant wheat cultivars. Some were serologically related to the PR proteins, chitinases, and were suspected to be involved in resistance to the RWA (Van der Westhuizen and Pretorius 1996). Peroxidases are also implicated in several defence-related events that occur in the extracellular matrix (Bowles 1990).

The purpose of the study reported here was to ascertain whether intercellular chitinase and peroxidase activities are induced by RWA infestation in near-isogenic susceptible and resistant wheat cultivars and, if so, whether these activities are also induced systemically. Finally, we wanted to establish the effect of the genetic background, in which the resistance gene was bred, on the resistance response.

Materials and methods

Resistant wheat plants (*Triticum aestivum* L.) [cvs. 'Tugela DN', 'Betta DN', 'Molopo DN' (Gariep)] containing the *Dn-1* resistance gene from PI 137739 (Du Toit 1989a) and susceptible ones (cvs.

'Tugela', 'Betta', 'Molopo') to the RWA were grown under greenhouse conditions at a day temperature of 22 °C (±2 °C) and a night temperature of 16 °C (±2 °C). Culture conditions and infestation procedures are described by Du Toit (1988). Plants were infested (approx. 6 aphids per plant) at an early two leaf-stage by tapping a vial containing a predetermined number of aphids and thus scattering them onto the plants. The aphids multiply and as the plant develops, they infest younger leaves. Plants were harvested (excluding the first leaf) at specific times indicated and frozen immediately in liquid nitrogen. On the last harvesting day (14 days after infestation) plants of all cultivars were on average in the four- and early five-leaf growth stage.

To investigate the systemic induction of chitinase and peroxidase we caged approximately 30 aphids on the second leaves of 'Tugela DN' plants in the third-leaf growth stage. After 8 days of infestation the second and remaining leaves were harvested separately for assay purposes.

Antisera

Antisera against tobacco chitinase (PR-Q) (Stintzi et al. 1993) and horseradish peroxidase (Sigma) were used. Chitinases are serologically closely related between plant species and even between different plant families (Joosten and De Wit 1989).

Collection of intercellular fluids

At predetermined time intervals entire plants were harvested, after which the leaves were cut into 7-cm pieces. The cut ends were rinsed twice with distilled water. Intercellular wash fluids (IWF) were collected twice from the same leaf pieces by centrifugation (500 *g*) after vacuum infiltration (5 min) with 50 m*M* TRIS-HCl buffer (pH 7.8) and removal of excess liquid from the leaf surfaces.

Protein concentration

The protein concentration was determined according to the method of Bradford (1976) using bovine γ-globulin as a standard.

Malate dehydrogenase (MDH) activity

The relative activity of the cytosolic enzyme, MDH, in the apoplast was taken as a measure of cytosolic contamination.

Determination of MDH activity was according to Cooper (1977). The assay mixture contained 3.75 m*M* 1,4-dithiothreitol, 7 m*M* $MgCl_2$, 0.25 m*M* NADH, 2.3 m*M* oxalacetic acid in 80 m*M* phosphate buffer (pH 7.5) and an aliquot of crude enzyme (IWF or cell extract). The reaction was initiated by the addition of oxalacetic acid and monitored at 340 nm. Contamination was determined by expressing MDH activity in the apoplastic fluid as a percentage of the total (apoplastic and symplastic) MDH activity.

Peroxidase activity

A modified method of Zieslin and Ben-Zaken (1991) was used. The assay solution contained 0.1 *M* phosphate buffer (pH 5), 3 m*M* H_2O_2, 3 m*M* guaiacol and an aliquot of the enzyme extract. The formation of tetraguaiacol was monitored at 470 nm. Peroxidase activity was expressed as μ mol tetraguaiacol min^{-1} mg^{-1} protein. Sodium phosphate buffers of different pHs were used to determine the pH for optimum peroxidase activity.

Chitinase activity

The method of Boller et al. (1983) was modified. The initial reaction mixture (500 μl) containing 1.5 mg colloidal chitin in 50 μl sodium acetate buffer (pH 6.5) and an aliquot of enzyme extract was incubated at 37 °C for 30 min. After centrifugation (1000 *g*, 2 min) 0.3 ml of the supernatant was used in a second reaction mixture containing 20 μl 1.5% (m/v) desalted snail gut enzyme (cytohelicase) and 30 μl 1 *M* phosphate buffer (pH 7.1). Chitin oligomers formed in the first reaction were hydrolysed at 37 °C for 30 min after which the resulting GlcNAc was determined. A mixture of 0.25 ml secondary reaction solution and 50 μl 0.8 *M* tetraborate buffer (pH 9.1) was heated in a boiling water bath for 3 min. After cooling and addition of 1.5 ml 1% (m/v) 4-dimethyl-aminobenzaldehyde, the mixture was incubated at 37 °C for 20 min and subsequently cooled in tap water. The absorbance was read at 585 nm. A standard curve relating A_{585nm} to GlcNac concentration was used to calculate chitinase activity, which was expressed as μmol GlcNAc h^{-1} mg^{-1} protein. Sodium acetate buffers of different pHs were used to determine the pH for optimum chitinase activity.

Immunoblotting

Polypeptides of intercellular wash fluids were separated on SDS-PAGE gels in a mini-gel system according to Laemmli (1970). Western blots were performed by transferring the separated polypeptides onto nitrocellulose membranes using a BioRad Trans-Blot semidry Electrophoretic Transfer Cell. The transfer buffer contained 25 m*M* TRIS-HCl (pH 8.3), 192 m*M* glycine and 20% (v/v) methanol. The nitrocellulose filters were blocked for 1 h in 8% (m/v) bovine serum albumin in TBST buffer [10 m*M* TRIS-HCl, pH 7.9; 150 m*M* NaCl; 0.1% (v/v) Tween-20]. The blots were then incubated in primary antibody. The peroxidase and chitinase antisera were used at dilutions of 1 : 80 000 and 1 : 5000, respectively. After rinsing in TBST, the filters were probed with secondary antibody, goat antirabbit IgG (BioRad) at a dilution of 1 : 9000 in TBST containing 4% (m/v) BSA. After successive rinses in TBST and TBS (10 m*M* TRIS-HCl, pH 7.9; 150 m*M* NaCl), peroxidases and chitinases were detected using 0.018% 4-chloro-1-naphthol in phosphatase buffer (50 m*M* TRIS-HCl, pH 9; 50 m*M* $MgCl_2 \cdot 6H_2O$).

Results and discussion

Damage symptoms became visible approximately 10 days after infestation and had no visual effect on the growth rate of resistant and susceptible cultivars after 14 days of infestation. On a damage scale of one to six (Du Toit 1988), 'Molopo DN' (Gariep) and 'Betta DN' were each rated 3 and 'Tugela DN' 2.4 after 3 weeks of infestation. The rating is based on the sizes of chlorotic spots on the leaves: 1 = small isolated chlorotic spots, highly resistant; 6 = severe white/yellow streaks, leaves tightly rolled, highly susceptible. In contrast to wounding, the damage caused by the RWA, a phloem feeder, is due to the effect of a phytotoxin, secreted during feeding, which results in early chloroplast breakdown in susceptible cultivars (Fouché et al. 1984; Burt and Burton 1992). Ultrastructural studies showed that limited chloroplast breakdown occurred in the leaf cells of resistant cultivars after feeding (results not shown). According to Belefant-Miller et al. (1994) RWA-infested resistant barley produced significantly more collapsed, auto fluorescent cells than did infested susceptible barley, which was typical of a hypersensitive cell death response. To what extent wounding (possibly caused by probing aphids) is responsible for the RWA resistance response was not investigated in the present study, but a previous study has shown that the expression of chitinase

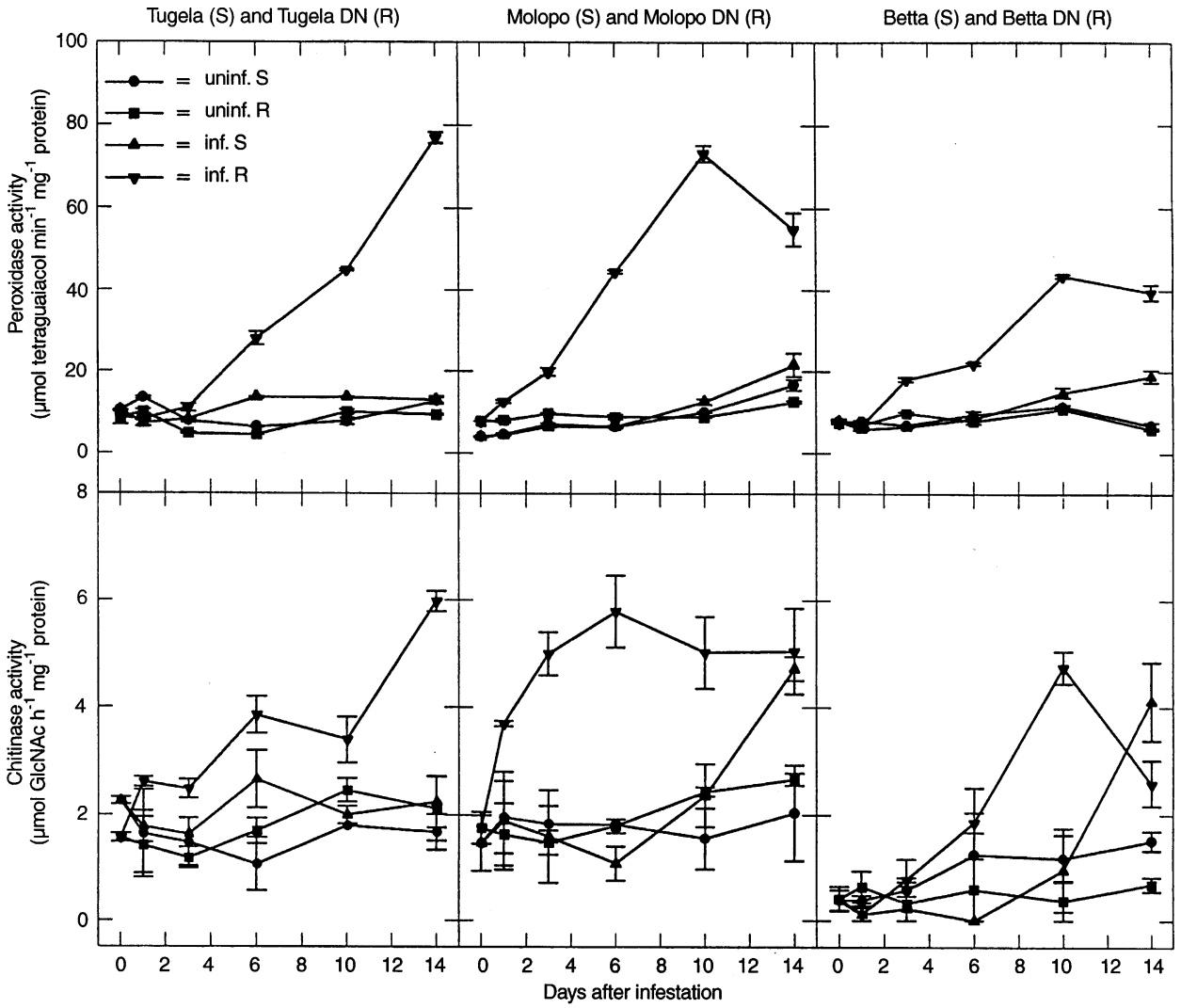

Fig. 1 Effect of RWA infestation (*inf.*) on intercellular peroxidase and chitinase activities of susceptible(*S*) and resistant (*R*) cultivars. Nonoverlapping standard deviations ($n = 3$) were regarded as significant differences

isoforms in response to mechanical wounding and RWA infestation was different (Botha et al. 1998).

No major cytosolic contamination of the IWF occurred as measured by the presence of MDH activity. The IWF MDH activity was below 0.1% of the total activity found in the cell. According to Fink et al. (1988), who used the same method of IWF collection from oat leaves, it is therefore unlikely that the cells were damaged.

The optimum pH for peroxidase activity was found to be 5.0 and the half-optimal pHs were 4.0 and 6.5. Maximum chitinase activity was at pH 6.5 and half maximum at 4.6. The intercellular peroxidases and chitinases are therefore well adapted to function in the acidic environment (pH 5.6) of the apoplast.

In all cultivars, the peroxidase activity in uninfested susceptible and resistant plants was very low and remained relatively constant during the investigation period with no significant differences (Fig. 1). Peroxidase activity was selectively induced by RWA-infestation to higher levels in all infested resistant cultivars. In contrast, no significant increase in peroxidase activity was observed in the infested susceptible cultivar, 'Tugela', while the peroxidase activity in the infested susceptible cultivars 'Molopo' and 'Betta' was significantly induced at a very late infestation stage. The increase in peroxidase activity in all infested resistant cultivars was initiated within 48 h of infestation. During the 14-day investigation period it reached different maximum levels in the different resistant cultivars, varying from five to tenfold higher than that of the control plants. This variation may be attributed to the genetic background in which the *Dn-1* resistance gene was bred. The lowest increase in activity was observed in 'Betta DN'. In a separate experiment where the peroxidase activity was followed in resistant 'Tugela' DN plants for 22 days of infestation, a 25-fold increase was observed (results not shown). No such data on this late stage of infestation are available for 'Molopo DN' and 'Betta DN'. When the activities were expressed per unit dry mass similar tendencies could be observed (results not shown).

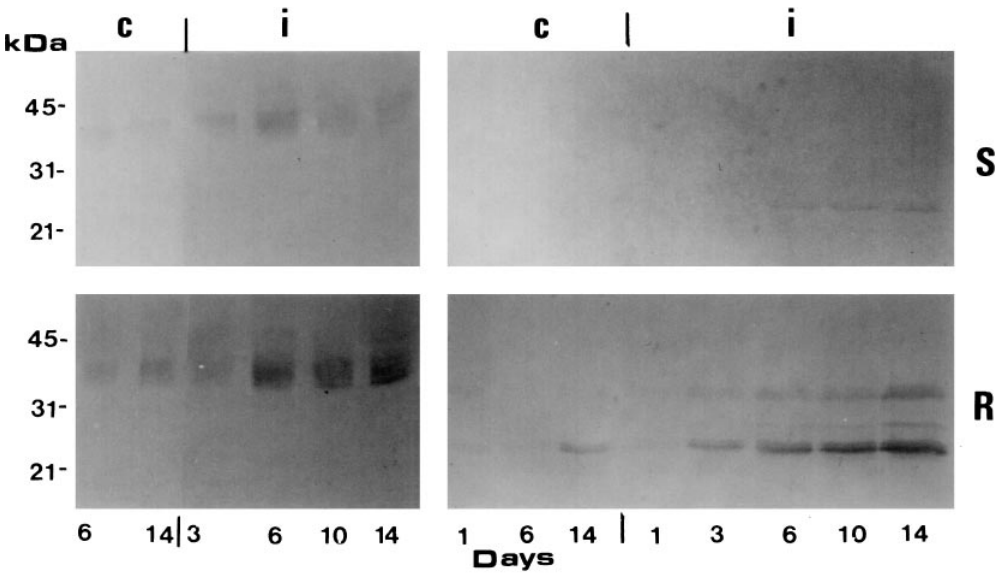

Fig. 2 Western blots of intercellular proteins, collected on different days during infestation, from uninfested (*C*) and infested (*i*) susceptible (*S*) cv 'Tugela' and resistant (*R*) cv 'Tugela DN'. Blots were probed with purified IgG against horseradish peroxidase (*left*) and antiserum against tobacco chitinase (PR-Q) (*right*)

There is quite a large number of reports on the induction of peroxidase activity by different biotic and abiotic stress conditions or treatment with elicitors. Most of these reports, however, deal with the nonselective induction of peroxidase by conditions other than insect attack (Bowles 1990). According to Gotthardt and Grambow (1992) Pgt-elicitor treatment resulted in a significant increase in peroxidase activity in a suspension culture of a resistant wheat line while the susceptible culture was unaffected. Nematode infestation of tomato plants selectively induced peroxidase activity in resistant cultivars (Zacheo et al. 1982). Ganguly and Dasgupta (1979) found only a slightly higher induction of peroxidase activity by nematodes in resistant tomato plants compared with susceptible ones. On our Western blots (Fig. 2) it can be seen that peroxidase-related proteins increased quantitatively as infestation proceeded, indicating that the increase in peroxidase activity was due to the induction of protein levels. Increased peroxidase activity not only occurred at the RWA feeding site (second leaves) but also in the uninfested leaves of the plant (Fig. 3). This systemic response was observed during wounding, virus, fungal or bacterial infections and infestation of roots with cyst nematodes (Bowles 1990).

The increased peroxidase activity in infested resistant wheat could be involved in an array of defence related reactions which collectively could contribute to resistance against the RWA. Peroxidases are involved in a few defence-related events that occur in the extracellular matrix. These include the strengthening of cell walls by lignification and the formation of intermolecular crosslinks, suberin formation and the production of reactive oxygen species, which is associated with eliciting and signalling events as well as direct defence (Bowles 1990; Mehdy 1994). In a separate investigation (results not shown) it was observed that RWA infestation, induced cell-wall thickening of approximately 12% in mesophyll cells of resistant wheat. This response likely hinders aphid probing. In several instances plant resistance to herbivores has been correlated with an enhanced oxidative state of the plant tissues (Felton et al. 1994) which involves the generation of reactive oxygen species. Oxidative shifts may result from the increased activities of oxidative enzymes such as lipoxygenase (Hildebrandt et al. 1989) or polyphenol oxidase (Felton et al. 1992) in addition to peroxidase (Bronner et al. 1991b). It is argued that the potential roles for oxidative injury in antiherbivore defence are: direct oxidative injury to the herbivore; indirect injury to the herbivore through oxidative damage to dietary compounds;

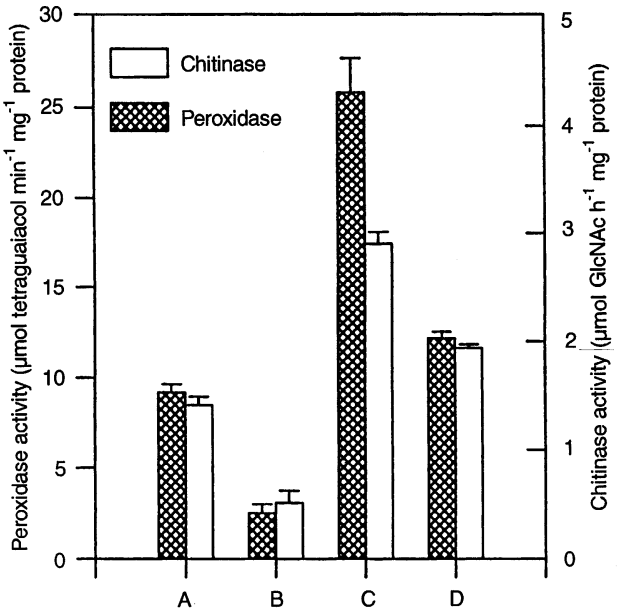

Fig. 3 Effect of RWA infestation of second leaves only on the intercellular peroxidase and chitinase activities of resistant ('Tugela DN') wheat leaves. *A* Uninfested second leaves (controls), *B* uninfested remaining upper leaves (controls), *C* infested second leaves, *D* infested remaining upper leaves. Nonoverlapping standard deviations ($n = 3$) were regarded as significant differences

signal transduction for eliciting plant defensive systems (Felton et al. 1994).

The chitinase activities in uninfested resistant and susceptible cultivars were low and remained relatively constant during the investigation period. RWA infestation selectively induced chitinase activity in all three resistant cultivars. The induction was initiated within 48 h of infestation. In fact, in 'Tugela DN' and 'Molopo DN' the increase was already evident 24 h after infestation. The maximum level reached in infested 'Betta DN' during the fourteen day investigation periods was 20% lower than in 'Tugela DN'. After fourteen days of infestation the chitinase activity of 'Tugela DN' was increased threefold (Fig. 1) and reached a peak (sevenfold increase) after 18 days (results not shown). When the activities were expressed per unit dry mass, similar tendencies were also observed (results not shown). There was a tendency for the chitinase activity of infested susceptible cultivars, especially 'Molopo' and 'Betta', to increase around the end of the investigation period (Fig. 1). This might be an indication that the chitinase-related defence response in these two susceptible cultivars is too late for effective resistance.

In *Solanum dulcamara* another group of phytophagous organisms, mites, also induce enhanced chitinase activity only in resistant cultivars (Bronner et al. 1991 b). There are also reports on the differential induction of chitinase activity between susceptible and resistant plants by pathogens and elicitors. Many examples are cited of the non-discriminatory induction of chitinase activity (Bowles 1990). Boyd et al. (1994) recorded the induction of chitinase gene expression in barley infected with powdery mildew. Fink et al. (1988) confirmed the presence of a considerable amount of chitinase in the intercellular space of oat crown rust-infected oat leaves.

According to the Western blots (Fig. 2), polypeptides serologically related to chitinase were selectively induced by RWA infestation to greater quantities in resistant than in susceptible wheat. These proteins distinctly increased quantitatively as infestation proceeded. This observation is in agreement with the results found for the chitinase activities (Fig. 1) and indicates that the increased activity can be ascribed to higher protein levels and not the activation of existing chitinases. The induction of chitinase activity not only occurred locally at the infestation site but also occurred systemically in other parts of the plant. The local activity was, however, higher than the remote activity (Fig. 3).

There is no clearly defined defence functions for chitinases against insect attack. There is, however, substantial evidence that chitinases indeed play a role in defence against microbial attack in vivo (Boijsen et al. 1993). It is suggested that citrus chitinases may play a role in defence against the borer weevil by disrupting the peritrophic membrane in the midgut (Mayer et al. 1995). However, many fluid-feeding insects, such as Homoptera are exceptional in lacking a peritrophic membrane (Gullan and Cranston 1994). Bronner et al. (1989) observed that feeding mites released some chitosan in plant cells, which presumably denaturated the DNA. Chitinases with chitosanase activity might remove these chitosans by hydrolysis, thereby protecting the plant cell DNA (Bronner et al. 1991 a). It is not known whether probing RWA release chitinous compounds. In addition, the digestion of such chitinous compounds by chitinases will produce oligosaccharide fragments, which might act as elicitors to trigger defence reactions (Osswald 1994). Another viewpoint is that increased chitinase activity might just be part of a general defence mechanism, e.g. hypersensitive reaction, which eventually is responsible for resistance. Belefant-Miller et al. (1994) indicated that the response to the RWA in resistant plants is similar to the hypersensitive response.

Results obtained in this study confirm those of previous studies of Van der Westhuizen and Pretorius (1995, 1996) that an effective RWA resistance response is induced by RWA infestation and not constitutively expressed. All indications are that increased intercellular peroxidase and chitinase activities are part of the resistance response. The RWA probes in the apoplast, which is recognised as the site at which the signals originate to elicit defence responses and where many defence-related products accumulate (Bowles 1990). As indicated, peroxidases might change the intercellular matrix in a number of ways. These changes include the strengthening of the cell walls and generation of reactive oxygen species which might be involved in eliciting and signalling events leading to an exaggerated resistance response. In addition, the reactive oxygen species might affect the RWA detrimentally as found by Felton et al. (1994) for other herbivores. No specific role in defence against aphids can be assigned to the PR-like chitinases as in the case of pathogenesis. These changes in enzyme activities in infested resistant cultivars might be part of one of the most important defence mechanisms in plants, the hypersensitive reaction (HR), which is elicited by different biotic stresses (Collinge et al. 1994). Noteworthy is that these enzyme activities were induced in all three resistant cultivars which were genetically different except for the presence of the *Dn-1* resistance gene. The level of response varied in the different resistant cultivars according to their resistance response in the field. The genetic background in which the *Dn-1* resistance gene is bred, therefore, plays a role in the effectiveness of the resistance response.

References

Belefant-Miller H, Porter DR, Pierce ML, Mort AJ (1994) An early indicator of resistance in barley to Russian wheat aphid. Plant Physiol 105: 1289–1294

Boijsen K, Nielsen KK, Gottschalk T, Mikkelsen JD (1993) Genetic transformation *Nicotiana benthamiana* with chitinase and β-1,3-glucanase genes from *Beta vulgaris* (sugar beet). In: Fritig B, Legrand M (eds) Mechanisms of plant defense responses. Kluwer Academic Publ, Dordrecht, The Netherlands, pp 449–510

Boller T, Gehri A, Mauch F, Vögeli U (1983) Chitinase in bean leaves: induction by ethylene, purification, properties and possible function. Planta 157: 22–31

Botha A-M, Nagel MAC, Van der Westhuizen AJ, Botha FC (1998) Chitinase isoenzymes in near-isogenic wheat lines challenged with Russian wheat aphid, exogenous ethylene and mechanical wounding. Bot Bull Acad Sin 39: 99–106

Bowles DJ (1990) Defense-related proteins in higher plants. Annu Rev Biochem 59:873–907

Boyd LA, Smith PH, Green RM, Brown JKM (1994) The relationship between the expression of defense-related genes and mildew development in barley. Mol Plant-Microbe Interact 7:401–410

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Bronner R, Westphal E, Dreger F (1989) Chitosan, a component of the compatible interaction between *Solanum dulcamara* L. and the gall mite *Eriophyes caldophthirus* Nal Physiol Mol Plant Pathol 34:117–130

Bronner R, Westphal E, Dreger F (1991a) Pathogenesis-related proteins in *Solanum dulcamara* L. resistant to the gall mite *Aceria cladophthirus* (Nalepa) (syn *Eriophyes cladophthirus* Nal.). Physiol Mol Plant Pathol 38:93–104

Bronner R, Westphal E, Dreger F (1991b) Enhanced peroxidase activity associated with the hypersensitive response of *Solanum dulcamara* to the gall mite *Aceria cladophthirus* (Acari: Eriophyoidea). Can J Bot 69:2192–2196

Burt JD, Burton RL (1992) Characterization of plant damage caused by Russian wheat aphid (Homoptera: Aphididae). J Econ Entomol 85(5):2017–2022

Collinge DB, Gregersen PL, Thordal-Christensen H (1994) The induction of gene expression in response to pathogenic microbes. In: Basra AS (ed) Mechanisms of plant growth and improved productivity. Marcel Dekker, New York, pp 391–432

Cooper TG (1977) The tools of biochemistry. John Wiley and Sons, New York

Du Toit F (1988) A greenhouse test for screening wheat seedlings for resistance to the Russian wheat aphid, *Diuraphis noxia* (Homoptera: Aphididae). Phytophylactica 20:321–322

Du Toit F (1989a) Inheritance of resistance in two *Triticum aestivum* lines to the Russian wheat aphid (Homoptera: Aphididae). J Econ Entomol 82:1251–1253

Du Toit F (1989b) Components of resistance in three bread wheat lines to Russian wheat aphid (Homoptera: Aphidiae) in South Africa. J Econ Entomol 82:1779–1781

Felton GW, Workman J, Duffey SS (1992) Avoidance of antinutritive plant defense: Role of midgut pH in Colorado potato beatle. J Chem Ecol 18:571–583

Felton GW, Bi JL, Summers CB, Mueller AJ, Duffey SS (1994) Potential role of lipoxygenases in defense against insect herbivory. J Chem Ecol 20:541–666

Fink W, Liefland M, Mendgen K (1988) Chitinases and β-1,3-glucanases in the apoplastic compartment of oat leaves (*Avena sativa* L.) Plant Physiol 88:270–275

Fouché A, Verhoeven RL, Hewitt PH, Walters MC, Kriel CF, De Jager J (1984) Russian aphid (*Diuraphis noxia*) feeding damage on wheat, related cereals and a *Bromus* grass species. In: Walters MC (ed) Progress in Russian wheat aphid (*Diuraphis noxia*) Mordw. research in the Republic of South Africa. Tech Commun Dep Agric Repub S Afr No 191:22–33

Ganguly AK, Dasgupta DR (1979) Sequential development of peroxidase (EC 1.11.1.7) and IAA-oxidase activities in relation to resistant and susceptible responses in tomatoes to the root-knot nematode, *Meloidogyne incognita*. Indian J Nematol 9:143–151

Gotthardt U, Grambow HJ (1992) Near-isogenic wheat suspension cultures: establishment, elicitor induced peroxidase activity and potential use in the study of host pathogen-interactions. J Plant Physiol 139:659–665

Gullan PJ, Cranston PS (1994) The insects: an outline of entomology. Chapman and Hall, London

Hildebrand DF, Rodriguez JG, Legg CS, Brown GC, Bookjans G (1989) The effects of wounding and mite infestation on soybean leaf lipoxygenase levels. Z Naturforsch 44:655–659

Joosten MHAJ, De Wit JGM (1989) Identification of several pathogenesis-related proteins in tomato leaves inoculated with *Cladosporium fulvum* (syn. *Fulvia fulva*) as 1,3-β-glucanases and chitinases. Plant Physiol 89:945–951

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature 227:680–685

Mayer RT, Shapiro JP, Berdis E, Hearn CJ, McCollum TG, McDonald, RE, Doostdar H (1995) Citrus rootstock responses to herbivory by larvae of the sugarcane rootstock borer weevil (*Diaprepes abbreviatus*). Physiol Plant 94:164–173

Mehdy MC (1994) Active oxygen species in plant defense against pathogens. Plant Physiol 105:467–472

Osswald WF, Shapiro JP, Doostdar H, McDonald RE, Niedz RP, Nairn, CJ, Hearn CJ, Mayer RT (1994) Identification and characterization of acidic hydrolases with chitinase and chitosanase activities from sweet orange callus tissue. Plant Cell Physiol 35:811–820

Pollard DG (1973) Plant penetration by feeding aphids (Hemiptera Aphidoidea): a review. Bull Entomol Res 62:631–714

Smith CM, Schotzko D, Zemetra RS, Souza EJ, Schroeder-Teeter S (1991) Identification of Russian wheat aphid (Homoptera: Aphididae) resistance in wheat. J Econ Entomol 84:328–332

Stintzi A, Heitz T, Prasad V, Wiedemann-Merdinoglu S, Kauffmann S, Geoffroy P, Legrand M, Fritig B (1993) Plant 'pathogenesis-related' proteins and their role in defense against pathogens. Biochimie 75:687–706

Van der Westhuizen AJ, Pretorius Z (1995) Biochemical and physiological responses of resistant and susceptible wheat to Russian wheat aphid infestation. Cereal Res Commun 23:305–313

Van der Westhuizen AJ, Pretorius Z (1996) Protein composition of wheat apoplastic fluid and resistance to the Russian wheat aphid. Aust J Plant Physiol 23:645–648

Walters MC, Penn F, Du Toit F, Botha TC, Aalbersberg K, Hewitt PH, Broodryk SW (1980) The Russian wheat aphid. Farming S Afr, Leaflet Ser, Wheat G3, 1–6

Zacheo G, Bleve-Zacheo T, Lamberti F (1982) Role of peroxidase and superoxidase dismutase activity in resistant and susceptible tomato cultivars infested by *Meloidogyne incognita*. Nematol Mediterr 10:75–80

Zieslin N, Ben-Zaken R (1991) Peroxidase, phenylalanine ammonia-lyase and lignification in peduncles of rose flowers. Plant Physiol Biochem 29:147–151