RESISTANCE TO *ELDANA SACCHARINA* (LEPIDOPTERA: PYRALIDAE) IN SUGARCANE AND SOME PHYTOCHEMICAL CORRELATIONS

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Abstract

The sugarcane borer, *Eldana saccharina* Walker is a serious pest of sugarcane in South Africa. Eldana survival and growth are influenced by sugarcane varieties and resistance has been correlated with high fibre and tannin contents. There are, however, exceptions, e.g. N19 is more resistant than its fibre and tannin contents would suggest. Resistance can also be linked with bud scale chlorogenate and flavonoid patterns. Near infra-red (NIR) detectable components of stalk surface wax appear to be more highly correlated with resistance to eldana than any other trait determined in this study. After calibration of an NIR spectrophotometer with wax from a series of standard varieties of known eldana resistance ratings, predictions of resistance for unreleased varieties can be made. This represents the first such use for the powerful technique of NIR spectroscopy. However, for all five traits examined, no cause and effect relationships are necessarily implied. Implications for the sugarcane breeding programme are discussed.

Introduction

The use of resistant varieties for the control of borers in sugarcane is a promising component of any integrated pest management system (Brader, 1979). Nuss *et al.* (1986) found that correlations between *Eldana saccharina* Walker numbers and brix % dry matter in field trials were positive and significant ($p < 0,05$) indicating that varieties with high sucrose and low fibre tend to be susceptible. However, such varieties for obvious reasons, are otherwise highly desirable. Correlation coefficients between eldana numbers and brix % dry matter were generally low to moderate ($r^2 = 0,15$ to $0,56$), leading these authors to suggest that the selection of resistant varieties of low fibre and high sucrose contents might be possible.

The purpose of the research reported here was to identify resistance mechanisms which potentially have a low cost in terms of reduction in final sucrose yield. High fibre can be considered to be at high cost (Penning de Vries *et al.* 1974; Lambers and Rychter, 1989). It was therefore necessary to identify and obtain data on as many putative resistance traits as possible, of both the less desirable and desirable types.

Controlled inoculation trials, intended to distinguish between resistant and susceptible varieties, are costly, long term and notoriously prone to yield few significant results, unless repeated many times (*Murdoch, personal communication). It was therefore decided that the listed eldana resistance ratings for commercial varieties would be used in attempts to correlate them with phytochemical characteristics. These ratings are expressed as eldana numbers per 100 stalks, where NCo376 has been assigned a rating of 100, and are based on several long term variety trials.

It has been asserted in the past that eldana numbers are decided within the first week after egg hatching, and that during this time first instar larvae are still on the stalk surface (**Nuss, personal communication). Consequently it is possible that plant surface chemistry might be involved in resistance to eldana. Since sugarcane stalks are often covered in a copious bloom of wax, it was decided to investigate this aspect. Woodhead (1987) and Woodhead and Padgham (1988) have found that the surface wax of sorghum and rice contributes towards the resistance of these plants to insect pests.

Another aspect of surface chemistry is that of epidermal flavonoid content. Early in the eldana epidemic it was noticed that amongst the then current varieties, those that were smut resistant were generally eldana susceptible. Lloyd and Pillay (1980) correlated increasing bud scale flavonoid contents with smut resistance. Since eldana may enter the stalk via buds (Leslie, 1993), it was decided to investigate budscale flavonoids in relation to eldana resistance. Flavonoids have been implicated in other examples of host plant resistance; for example, in the resistance of maize to the corn earworm *Helicoverpa zea* (Boddie) (Snook *et al.* 1989; Wiseman *et al.* 1992). Williams and Harborne (1977) have found that the plant families Cyperaceae, Juncaceae and Gramineae, all of which contain host-plant species for eldana (***Conlong, personal communication), are closely related in terms of their flavonoid chemistry.

Methods

Fibre, tannin and lignin

Fibre contents were determined gravimetrically on mature internode samples following neutral detergent extraction (Hartley, 1978). Lignin, as a percentage of fibre, was determined by NIR spectroscopy, from fibre that had been extracted and solvent leached according to Hartley (1978). The instrument was calibrated using gravimetric Klason lignin determinations as outlined by Garbutt *et al.* (1988). Results were expressed as a percentage of NCo376 .

The following colorimetric method of condensed tannin analysis was developed based on the method of Bate-Smith (1977). Internode cross sections of 10 g fresh mass were macerated and incubated at 50°C for 4 h in 50 ml of methanol containing 5% v/v of concentrated HCl. Samples were then filtered and the absorbance of the methanol extract was read at 494 nm. Results were again expressed as a percentage of NCo376.

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Flavonoids and chlorogenates

Both immature and mature bud scales were sampled. Fresh masses of 0,2 g and 0,4 g respectively, were extracted in 3 ml methanol, 1 ml chloroform and 1 ml water by heating at 80°C for 10 mins in a sealed vial followed by maceration. Macerates were then filtered through Whatman # 1 paper, cooled, and rinsed with 2 ml 50% (v/v) chloroform:methanol and 2 ml 50% (v/v) methanol:aqueous 200 mM phosphate buffer pH 8 sequentially. Extra chloroform was added to the filtrate which was then chilled until two layers of equal volume formed. The upper aqueous:methanol layer was recovered, acidified and dried under nitrogen. The dry extract was then redissolved in 200 μl of methanol followed by 300 μl of water. Twenty μl were separated by high performance liquid chromatography (HPLC) after 0,22 μm filter tip filtration. HPLC conditions were as follows: Column C18; gradient from 90% solvent A to 50% solvent B in 50 minutes (A = water altered to pH 2 with H_3PO_4: B = 7:3 acetonitrile: methanol). Detection 345 nm; column temperature 35°C and column flow rate 1 ml/minute.

Surface wax

Waxes from mature stalks of a series of commercial varieties of known eldana ratings were dissolved in carbon tetrachloride in the ratio of 0,1 g per 10 ml. Thin layers under slides were analysed by a filter based NIR spectrophotometer (Technicon 450) interfaced to a personal computer. Absorption bands between 1 000 and 2 500 nm were recorded as the log of the reciprocal of the reflectance (R) at each filter wavelength. The principles of the technique are described by Meyer and Wood (1988).

A multiple regression analysis was performed in which a set of filters was chosen stepwise by computer. Weighting factors (F) were allocated to these wavelengths. Equations produced for the prediction of eldana per 100 stalks (e/100 st), had the following form:

Predicted e/100 st = $F_0 + (F_1 \times \log 1/R_1) + (F_2 \times \log 1/R_2)$ .... etc.

Series of commercial varieties were sampled over several months (8 month old to 12 month old cane).

Unknowns and varieties of known eldana ratings were grown in pots for 12 months prior to inoculation with eldana eggs. Wax samples were taken by stalk scraping and eldana numbers were assessed by stalk splitting some 500 day-degrees later (*Leslie, personal communication).

Wax samples from further series of commercial varieties were prepared for analysis with a scanning NIR instrument. This instrument is not limited to individual narrow bands as are filter based instruments, and it is possible to achieve higher correlation coefficients, frequently with fewer wavelengths.

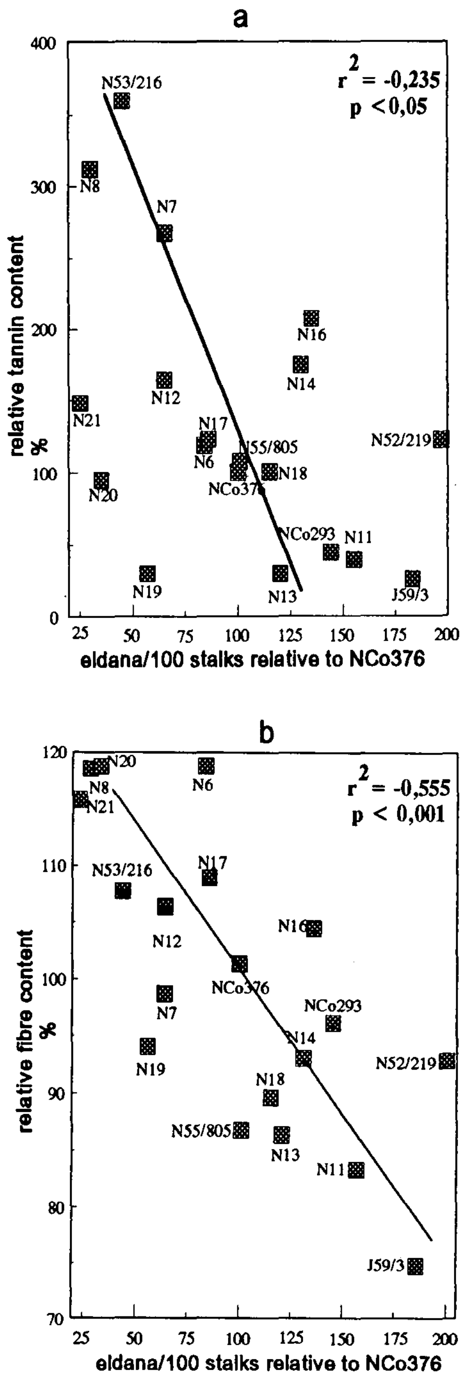
Results and Discussion

Fibre, tannin and lignin

The association of tannin content with eldana resistance was low but still significant (Figure 1a). Fibre correlated better with eldana resistance than tannin (Figure 1b). As suggested by Nuss *et al.* (1986) certain low fibre varieties were nevertheless resistant. For example N19, based on fibre content, would be predicted as susceptible to eldana. It would also be predicted as susceptible based on its tannin content. Lignin, as a percentage of fibre, showed no association with eldana resistance (r^2 = -0,018 p > 0,1) unless it was included with fibre in a multiple regression. When this was done increasing lignin content seemed to correlate with susceptibility. A model involving all three of these traits had the following form:

Predicted e/100 st = 112 + (-343 × Rel:fibre) + (344 × Rel:lignin) + (-14 × Rel:tannin).

Negative weighting factors are associated with resistance traits. The t-test of tannin content within the regression was not significant (p > 0,1), whilst those for lignin and fibre were significant (p < 0,02). Tannin is apparently of little value in a multiple regression due to its association with fibre (data not shown). Figure 2 shows the association between actual eldana ratings and those predicted by the tannin, lignin, fibre model.



FIGURES 1a and b Relationship between eldana ratings and a) internode relative tannin content, b) internode relative fibre content.

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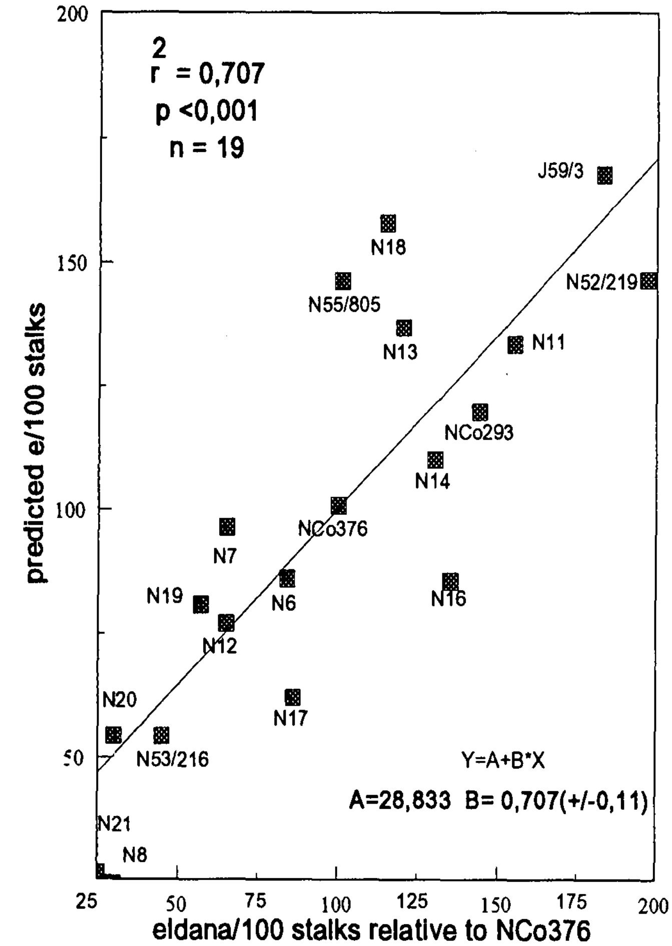


FIGURE 2 Relationship between eldana ratings and predicted ratings using a multiple regression of relative tannin, lignin and fibre contents.

Flavonoids and chlorogenates

Immature bud scales (upper stalk) from cane 12 months cane in April, were found to contain much higher levels of flavonoids than mature bud scales (lower stalk). However, varietal patterns were consistent between immature and mature bud scales (data not shown). Figures 3 and 4 are examples of flavonoid HPLC patterns for immature bud scales from a resistant variety (N21) and a susceptible variety (N11).

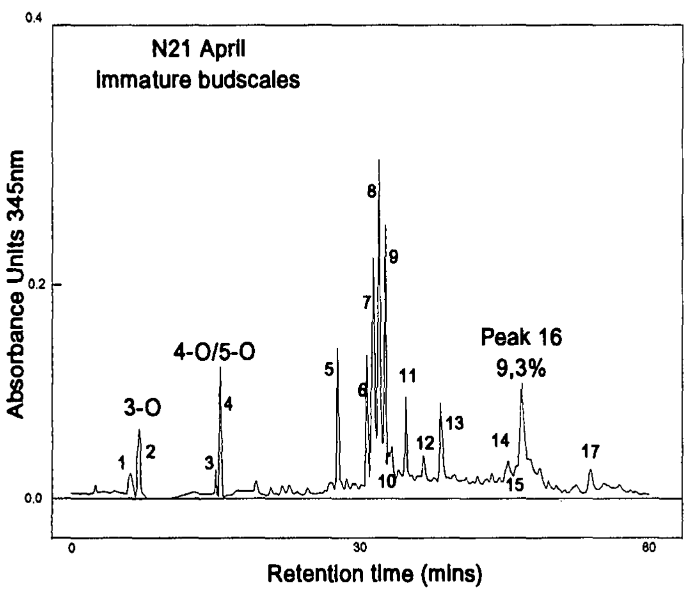


FIGURE 3 N21 flavonoid HPLC chromatogram from bud scales sampled from the top three immature nodes below the natural breaking point. Age of cane – 12 months. (3-O, 4-O and 5-O are three isomers of chlorogenic acid).

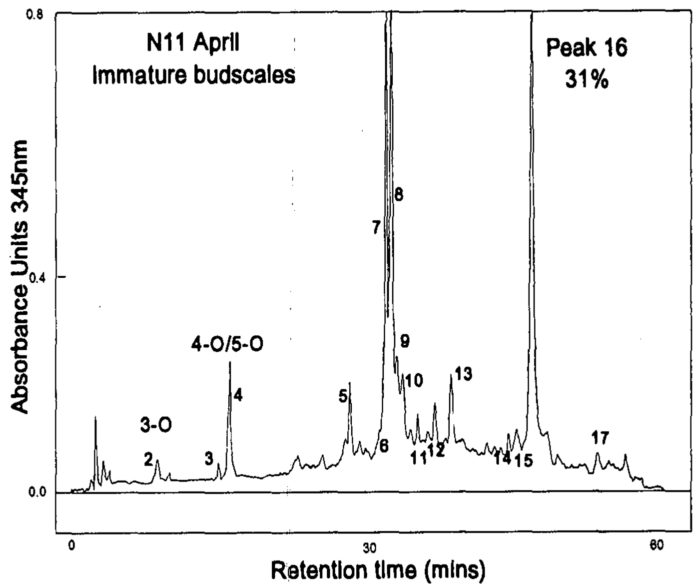
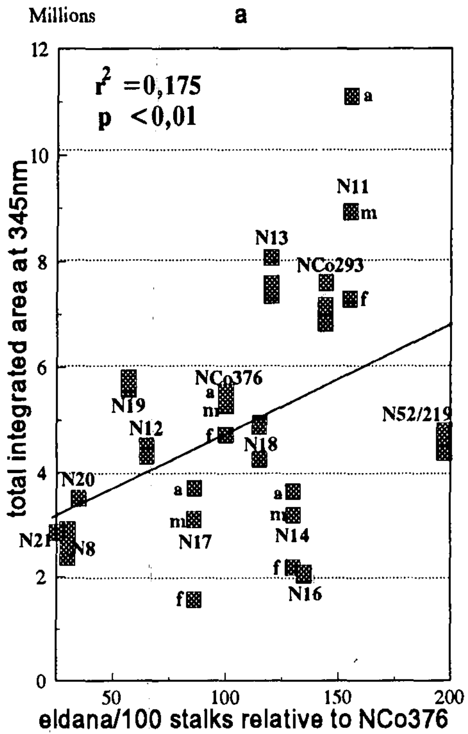


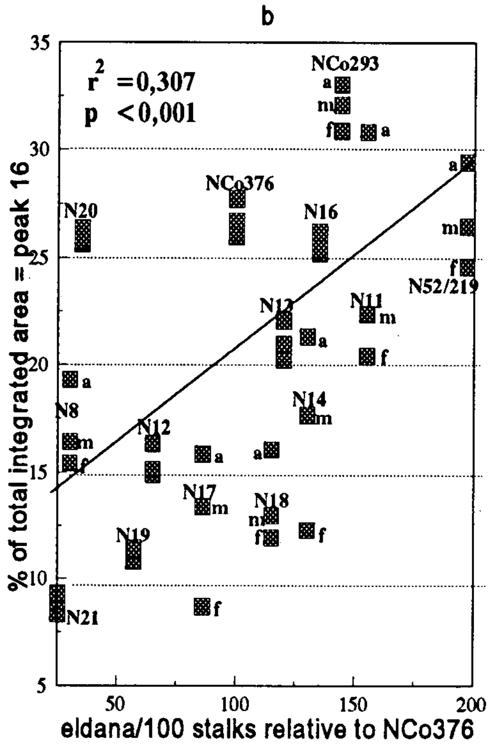
FIGURE 4 N11 flavonoid HPLC chromatogram from bud scales sampled from the top three immature nodes below the natural breaking point. Age of cane – 12 months. (3-O, 4-O and 5-O are three isomers of chlorogenic acid).

Analyses were repeated over three months for a series of commercial varieties. Figure 5a shows the association between total flavonoid content and variety eldana ratings. More significantly, there is a closer association between the proportion of total flavonoid content that is represented by peak 16 and variety eldana ratings (Figure 5b). There was a general trend for both total flavonoids and % peak 16 to increase over the three sampling months.

Figure 6 shows the association of predicted eldana ratings with actual ratings produced by a multiple regression of total flavonoids and % peak 16:

Predicted e/100 st = 9,47 + [3,23 × % peak 16] + [5,6 × total flavonoids].





FIGURES 5a and b Relationship between bud scale flavonoids and eldana ratings: a) total flavonoid content b) % of total = peak 16 (f,m,a - February, March and April).

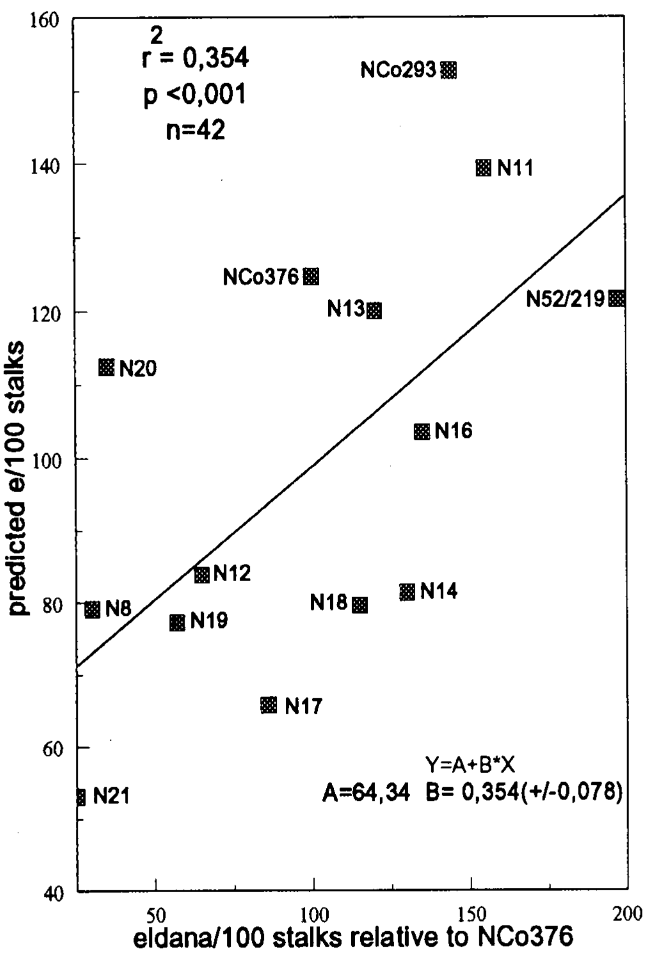


FIGURE 6 Relationship between actual eldana ratings and predicted ratings using a multiple regression of total and % peak 16 bud scale flavonoid contents. (Individual monthly data was used though only 3 months averages are shown).

Positive weighting factors are associated with susceptibility traits. The t-test of total flavonoid content within the regression was significant only at the 0,1 level of probability.

Increasing total flavonoid content and the percentage of the total represented by peak 16 appear to be associated with varietal susceptibility to eldana. Many examples in the literature, of host plant resistance involving flavonoids, associate increasing flavonoid content with resistance. For example, the resistance of maize to the corn earworm and the fall armyworm *Spodoptera frugiperda* (JE Smith) is associated with increasing contents of the flavonoid maysin (Wiseman *et al.* 1992). Whole and fractionated flavonoid extracts are being bio-assayed against eldana.

Surface wax

Due to the limited number of commercial varieties for which reliable eldana ratings were available, it was decided that as few NIR wavelengths as possible would be used in a multiple regression. Also these wavelengths should be repeatable between replicate series of commercial varieties. Typically, in other applications of NIR, three or four wavelengths (filters) are chosen and correlation coefficients (r^2) of around 0,9 are achieved. However, these are for directly measurable components such as total nitrogen content in soils (Dalal and Henry, 1986), sucrose in cane juice (Meyer and Wood, 1988) and lignin in fibre (Garbutt *et al.* 1988).

Good correlations between actual eldana ratings and those predicted using NIR on wax were achieved (Table 1). The overall association for six separate series of varieties is shown in Figure 7.

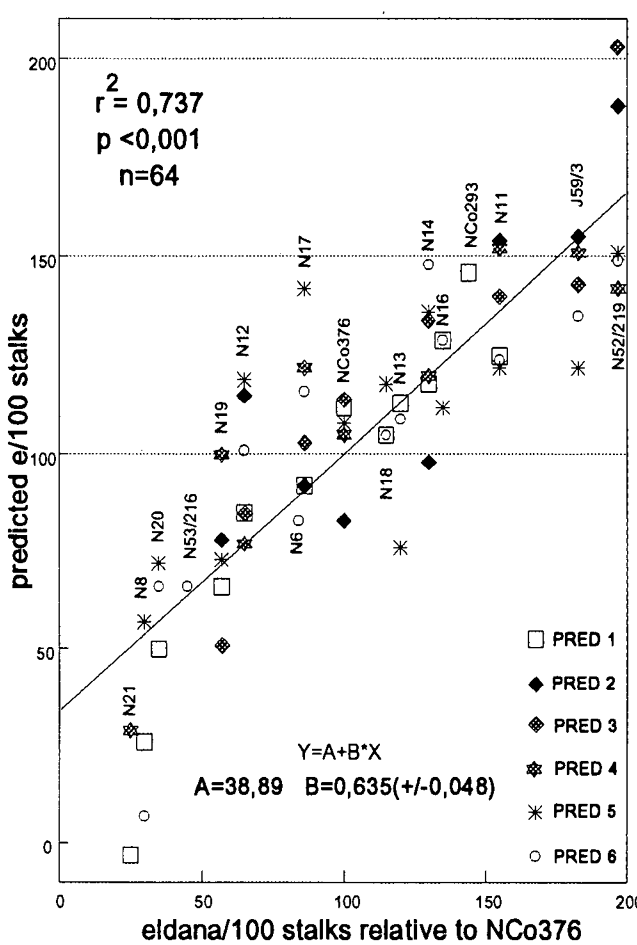


FIGURE 7 Relationship between actual eldana ratings and predicted ratings using the NIR reflectance data at three wavelengths on dissolved stalk surface wax.

Table 1

Filters, correlation coefficients and assigned weighting factors for near infra-red spectroscopy of stalk surface wax. Filter based instrument - normal mathematics

	(Site 1) Prediction 1 Age 8 months	(Site 2) Prediction 2 8 months	(Site 2) Prediction 3 9 months	(Site 2) Prediction 4 10 months	(Site 3) Prediction 5 12 months	(Site 4) Prediction 6 12 months
Number of varieties	13	8	9	9	13	14
Filters chosen:	F9 2139 nm F11 1982 nm F12 1818 nm	F9 2139 nm F11 1982 nm F13 1778 nm	F9 2139 nm F11 1982 nm F13 1778 nm	F9 2139 nm F11 1982 nm F12 1818 nm	F14 2100 nm F16 1940 nm F17 1734 nm	F9 2139 nm F11 1982 nm F12 1818 nm
r^2 =	**0,89** p < 0,001 s.e. = 17	**0,83** p < 0,005 s.e. = 29	**0,84** p < 0,001 s.e. = 27	**0,72** p < 0,005 s.e. = 39	**0,62** p < 0,005 s.e. = 39	**0,69** p < 0,001 s.e. = 34
Weighting factors are:	F0 − 1124 F9 + 190153 F11 − 310971 F12 + 124608	F0 − 3857 F9 + 214960 F11 − 337871 F13 + 133811	F0 − 2447 F9 + 136880 F11 − 243758 F13 + 100913	F0 + 314 F9 + 314496 F11 − 519106 F12 + 205671	F0 − 3936 F14 + 166593 F16 − 174963 F17 + 19713	F0 + 1414 F9 + 247415 F11 − 419475 F12 + 169533

For each series of varieties, computer based selection of filters was reasonably consistent (Table 1). Other filters were sometimes chosen, but these were close in wavelength to what could be considered as the first choice wavelengths (filters 9, 11 and 12). In the stepwise computer selection, filter 11, or an alternate, was chosen first in every case. Weighting factors for the three filters respectively, although variable, were consistently positive, negative and positive.

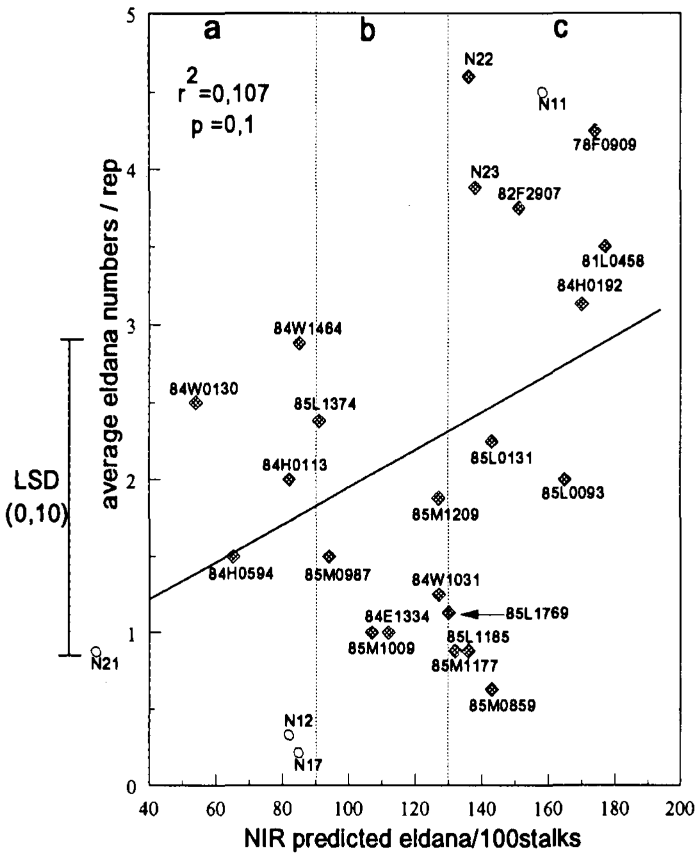


FIGURE 8 Relationship between NIR predicted eldana ratings (calibrated with 9 'unknowns') and eldana infestation levels in a pot trial for a series of inknowns. (Group 'a' is significantly different from group 'c'. Members of group 'b' are not significantly different from 'a' or 'c' or both; p = 0,1).

For validation experiments it is therefore necessary to include a set of known varieties for calibration.

Figure 8 shows the results of a validation pot trial in which a number of unreleased varieties from the breeding programme were tested against eldana. Of nine calibration varieties only N21, N12, N17 and N11 were included in the pot trial. The association was between average eldana numbers per replicate pot (n=8) and NIR predicted eldana ratings, based on the actual ratings of the calibration varieties. The calibration results were comparable with those for previous series of standard varieties (see Figure 7 and Table 1) (r^2 = 0,81; p < 0,001; s.e. = 32).

All the unreleased varieties in the 'a' group of Figure 8 were predicted more resistant than those in the 'c' group (p = 0,1). A large variation in eldana numbers between replicates per variety, coupled with a high standard error of the filter based calibration, might explain the low level of association shown in Figure 8. Table 2 indicates what might have been achieved in terms of calibration results if a scanning instrument had been available.

Table 2

Wavelengths, correlation coefficients and assigned weighting factors for near infra-red spectroscopy of stalk surface wax. Scanning instrument - 2nd derivative mathematics

	Prediction 1	Prediction 2	Prediction 3
Number of varieties	11	13	13
Wavelengths chosen	2026 nm 1962 nm 1914 nm 1886 nm	1952 nm	1864 nm 1956 nm
r^2	0,96 p < 0,001 s.e. = 14	0,90 p < 0,001 s.e. = 18	0,91 p < 0,001 s.e. = 18
Weighting factors are:	F_0 −28519 F_1 + 150352 F_2 − 386588 F_3 + 499925 F_4 + 209817	F_0 + 85 F_1 + 45628	F_0 + 45 F_1 − 17416 F_2 + 27986

For the scanning instrument, stepwise combination of wavelengths was stopped as soon as the r^2 reached 0,9 or higher and standard errors fell below 20 (Table 2). As with the filter based instrument, a wavelength in the mid to upper 1 900s was chosen first. This suggests that a component of wax most highly correlated with eldana resistance is active in this band. Of interest were the very high correlations achieved for the second and third sets of varieties, using only one and two wavelengths respectively. Goddu and Delker (1960) published a chart of spectra-structure correlations and more recent charts are supplied by manufacturers with their instruments. The use of one such chart suggests that carbonyl and/or alcohol components of wax may be important in relation to eldana resistance. Whole and fractionated waxes are being bio-assayed against eldana.

Conclusions

It seems that a large proportion of the variation in sugarcane varietal resistance to eldana can be explained in terms of the tannin, lignin, fibre model. There appears to be a linkage between high fibre, high tannin, low flavonoid and unknown wax components (data not shown); i.e. these putative resistance traits tend to appear together in the same variety (except for N19). This poses the following questions: Which of the traits is actually operative? Is the chemical basis of the wax prediction just an indicator of other components which are the real resistance traits?

The surprise findings of this investigation were the high levels of association of unidentified wax components and high flavonoid contents with variety eldana ratings. Of the traits determined, wax components appear most likely to have an *in-vivo* effect, due to a higher overall level of association with eldana ratings, than any other investigated trait. It is not claimed that any of the associations represent cause and effect. However, all of the methods employed seem to have predictive value. Through the use of NIR an indication as to the nature of likely active components in wax has been obtained.

Should waxes and flavonoids prove to be with effect in the sugarcane-eldana interaction and if any linkage with high fibre/tannin can be broken, as appears to be the case for N19, the selection of eldana resistant high sucrose varieties will become a more easily attainable goal.

Acknowledgments

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REFERENCES

Bate-Smith, EC (1977). A simple method for the colorimetric determination of condensed tannins. *Phytochemistry* 16: 1421-1426.

Brader, L (1979). Integrated pest control in the developing world. *Ann Rev Entomol* 24: 225-254.

Dalal, RC and Henry, RJ (1986). Simultaneous determination of moisture, organic carbon, and total nitrogen by near infra-red reflectance spectrophotometry. *Soil Sci Soc Am J* 50: 120-123.

Garbutt, DCF, Donkin, MJ and Meyer, JH (1988). Near infra-red reflectance analysis of cellulose and lignin in wood. *TAPPSA Congress* 1988.

Goddu, RF and Delker, DA (1960). Spectra-structure correlations for the near infra-red region. *Anal Chem* 32: 140-141.

Hartley, RD (1978). HPLC for the separation and determination of phenolic compounds in plant cell walls. In: Modern Methods of Plant Analysis. (Eds). HF Linskens and JF Jackson. Springer Verlag. pp 92-103.

Lambers, H and Rychter, AM (1989). The biochemical background of variations in respiratory rate: Respiratory pathways and chemical composition. In: Causes and Consequences of Variation in Growth Rate and Productivity of Higher Plants. *(Ed). H Lambers.* SPB Academic Publishing. pp 199-225.

Leslie, GW (1993). Dispersal behaviour of neonate larvae of the pyralid sugarcane borer *Eldana saccharina. Proc S Afr Sug Technol Ass* 67: (in press).

Lloyd, HL and Pillay, M (1980). The development of an improved method for evaluating sugarcane for resistance to smut. *Proc S Afr Sug Technol Ass* 54: 1-5.

Meyer, JH and Wood, RA (1988). Rapid analysis of cane juice by near infrared reflectance. *Proc S Afr Sug Technol Ass* 62: 203-207.

Nuss, KJ, Bond, RS and Atkinson, PR (1986). Susceptibility of sugarcane to the borer *Eldana saccharina* (Walker) and selection for resistance. *Proc S Afr Sug Technol Ass* 60: 153-155.

Penning de Vries, FWT, Brunsting, AHM and Van Laar, HH (1974). Products, requirements and efficiencies of biosynthetic processes: a quantitative approach. *J Theor Biol* 45: 339-377.

Snook, ME, Widstrom, NW and Gueldner, RC (1989). Reversed-phase high-performance liquid chromatographic procedure for the determination of maysin in corn silks. *J Chromatog* 477: 439-447.

Williams, CA and Harborne, JB (1977). Flavonoid chemistry and plant geography in the Cyperaceae. *Biochem System Ecol* 5: 45-51.

Wiseman, BR, Snook, ME, Isenhour, DJ, Mohm, JA and Widstrom, NW (1992). Relationship between growth of corn earworm and fall armyworm larvae (Lepidoptera:Noctuidae) and maysin concentration in corn silks. *J Econ Entomol* 85: 2473-2477.

Woodhead, S (1987). The influence of surface chemicals of sorghum on the behaviour of the stem borer *Chilo partellus* (Swinhoe). In Insects-Plants (Eds). V Labeyrie and D Lachaise. Junk Publishers. p 425.

Woodhead, S and Padgham, DE (1988). The effect of plant surface characteristics on resistance of rice to the brown planthopper, *Nilaparvata lugens. Entomol exp appl* 47: 15-22.