Relationships between Biomass and Phenolic Production in Grain Sorghum Grown under Different Conditions

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ABSTRACT

The total phenol pool (kg ha^{-1}) of the aerial parts and roots of sorghum (*Sorghum bicolor* L. cv. CE$_{145-66}$) crops and their relationships to dry matter and total phenol concentrations (g kg^{-1}) were studied in four different data sets (2 sites × 2 yr for a total of 52 smallholder fields) in Senegal. The total phenol pool size varied from 4 to 156 kg ha^{-1} in the aerial parts and from 1 to 16 kg ha^{-1} in the roots. The phenol pool size was closely correlated with the amount of dry matter in both the aerial parts (r = 0.95, P<0.001) and the roots (r = 0.91, P<0.001). In contrast, the phenol concentrations varied less between fields than the dry matter content, and therefore had less impact on the phenol pool size. Using a N nutrition index (NNI) to assess the N nutrition of the sorghum crops, both phenol the pool and concentration were higher when N nutrition was better. Grain yield and the phenol pool of aerial parts were also positively correlated. The data indicate that the environmental factors that promote growth and grain yield also enhance the total phenol synthesis in sorghum vegetative parts.

THE PRODUCTION OF PLANT PHENOLIC COMPOUNDS, which are thought to be part of the chemical defense of the plant, is under the control of both genetic and environmental factors. For instance, herbivore attacks (Tempel, 1981), insect damage (Woodhead and Bernays, 1978; Woodhead and Cooper-Driver, 1979; Guinn and Eindenbock, 1982), or reduced soil fertility (Cooper-Driver et al., 1977; Coley et al., 1985; Dustin and Cooper-Driver, 1992; Einhellig, 1996) will generally increase the synthesis of these compounds by the plants. This has been partly explained by the balance between C and nutrient availability (Bryant et al., 1983). In particular, N deficiency has been shown to strongly affect the synthesis of polyphenols (Koricheva et al., 1998) because it will affect growth more than photosynthesis, and thus allows more carbohydrates to be available for phenolic synthesis. These results have usually been obtained by determining the concentrations of total phenols or of different classes of compounds (e.g., tannins and phenolic acids) in plant tissues.

However, phenolic compounds are also known to have important effects once they are released from the living plant by directly or indirectly influencing soil quality and living organisms, including microorganisms and other plants. For instance, some agricultural plants like sorghum are known to release phenolic compounds such as sorgoleone (Einhellig and Souza, 1992; Einhellig et al., 1993; Weston et al., 1997) and phenolic acids (Ben-Hammouda et al., 1995; De Raissac et al., 1998) to the soil by decomposing crop residues (Patrick et al., 1963; Chou et al., 1981; Paul et al., 1994; Siqueira et al., 1991; Hoffman et al., 1996) and root exudates. Some of these compounds are shown to have negative impact on soil fertility and crops. An assessment of their potential toxic effects requires an estimation of the amount of polyphenolic compounds in the soil. This is related not only to the phenolic concentration in living tissue, but also to the absolute biomass production of the plant. Consequently, the total amounts of phenolic compounds accumulated in plants may be as important as their concentrations when determining the ability of plants to release allelochemicals.

In order to improve our understanding of the allelopathic potential of sorghum, we studied the variability of the phenolic production in its shoots and roots over a wide range of agricultural conditions in the Sahel zone of Senegal. An estimation of the total phenol concentration (g kg^{-1}) and pool size (kg ha^{-1}) was made based on dry matter data. The relationships between phenolic production, grain production, and N content (for the aerial parts) were established. In the areas investigated, the differences among fields in soil fertility, rainfall, and cropping systems are assumed to be large enough to result in differences in the available N and water for sorghum. This could lead to differences in both sorghum growth (Heron et al., 1963; Langlet, 1973; Ramu et al., 1991; Donatelli et al., 1992; Rego et al., 1998; Singh et al., 1998) and phenol accumulation.

MATERIALS AND METHODS

Measurements of the Plants

Four sets of smallholder fields cropped with sorghum CE$_{145-66}$ in the central peanut basin region of the Sahel area of Senegal were studied—two in 1996 and the other two in 1997. In both years, one set was in Sagnanème village (14°00′ N, 16°15′ W) and the other in Médina village (14°20′ N, 15°30′ W). The soils are ferralitic or ferruginous tropical soils with 61–78% sand in the 0- to 10-cm layer. The soil bulk density was not significantly different among fields and ranged from 0.147 to 0.160 kg m^{-3}. The pH ranged from 5.2 to 7.2 in the 0- to 40-cm layer. In 1996, 12 fields were studied in Sagnanème and 15 were studied in Médina. In 1997, eight other fields were studied in Sagnanème and 17 other fields were studied in Médina. No fertilizer, plant protection products, tilling, or irrigation were used, following the usual practice of local farmers. Weeds were removed by hand.

The sorghum was sown by the farmers at the beginning of the rainy season, which lasts from July to October. A 25- by

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Abbreviations: DW, dry weight; NNI, N nutrition index.

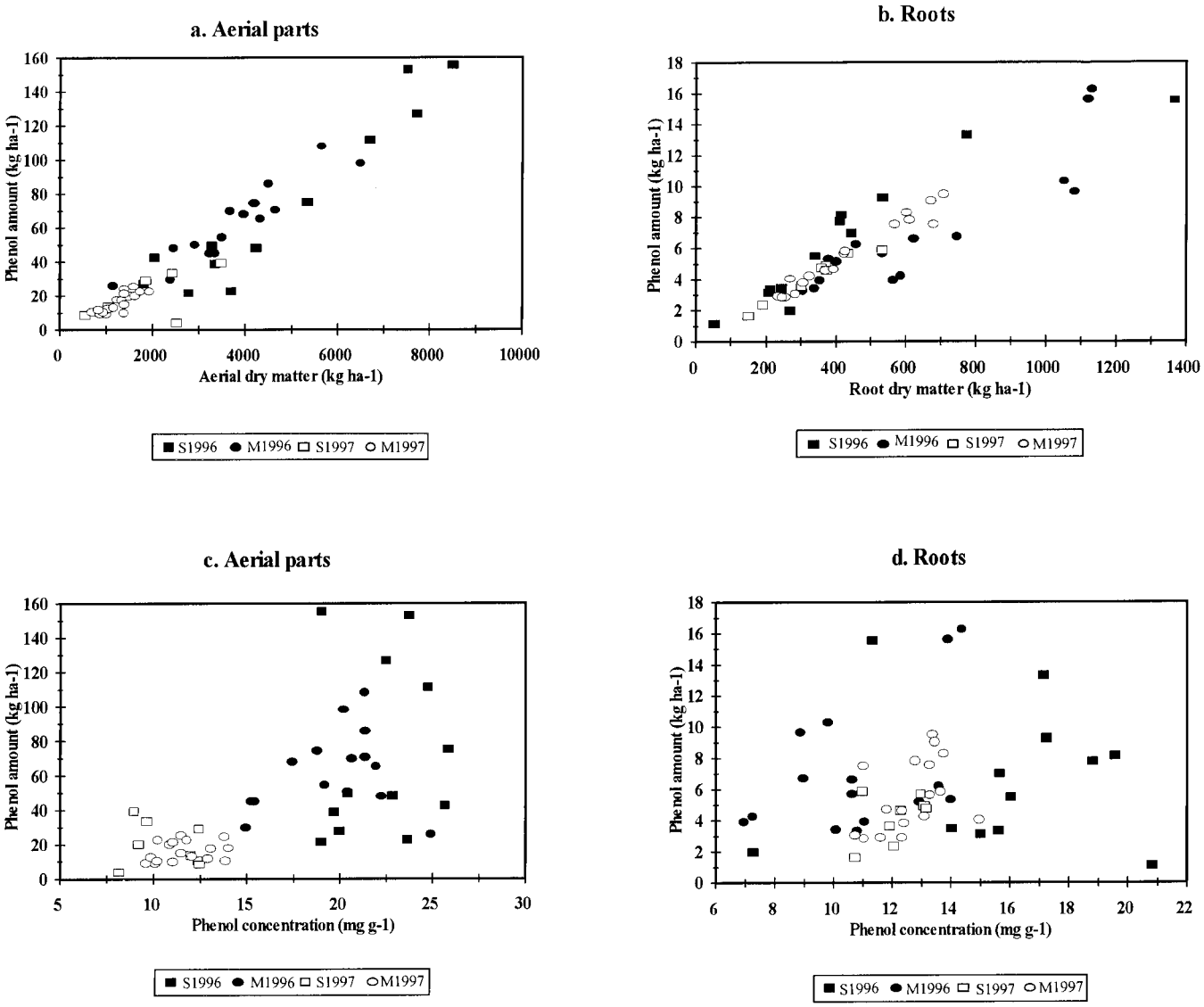

Fig. 1. Relationship between (*a*) the total phenol amount and dry matter of the aerial parts and (*b*) the total phenol amount and dry matter of the roots and between (*c*) the total phenol amount and conc. in the aerial parts and (*d*) the total phenol amount and conc. in the roots. S1996 and S1997: Fields at the Sagnanème site in 1996 and 1997; M1996 and M1997: Fields at the Médina site in 1996 and 1997.

25-m plot was marked in each field 15 d after crop emergence. Each plot was divided into 25 subplots that were 5 by 5 m. Five of these subplots were randomly chosen for growth and N controls, and another five were randomly selected for yield sampling. In each of the 25 subplots, the plants were counted and the between-row and within-row spacings were measured to determine the plant density so that the dry matter production and grain yields could be calculated.

In each field, 10 sorghum plants were randomly collected from each N control subplot at the flowering stage. This is the stage with the highest level of aerial and subterranean vegetative dry matter (Blondel, 1971; Vanderlip and Reeves, 1972) and the highest concentration of phenols (Woodhead, 1981). The aerial parts and roots of the plants were separated. The aerial parts from the 10 plants of each subplot were pooled to one sample per subplot, dried at 70°C for 96 h, weighed, and ground up. The five ground-up aerial tissue samples of each field were pooled, and a 50-g sample was taken for phenol analysis. The rest was used for N analysis, following the Bremner and Mulvaney (1982) method. Nitrogen concentration values are not available for 8 of the 15 fields in Médina in 1996. Root samples were treated in a similar way, but no N analysis was performed.

The aerial dry matter [kg ha^{-1} dry wt. (DW)] for each field was calculated as the mean dry matter of the five N control subplots. At the flowering stage, the root dry matter content was assessed in two subplots per field. In each subplot, 24 cylindrical cores of soil (total vol. of each core was 0.029 m^3 with a soil sampling vol. of 0.02 m^3) were taken horizontally just under a sorghum plant from the center of 24 squares (0.1 by 0.1 m) that corresponded to a hole measuring 0.6 m wide by 0.4 m deep. The samples were sifted and washed with water to separate the roots from the soil. The roots were dried at 70°C for 96 h and weighed. The root concentration (kg m^{-3}) in the soil was calculated for each subplot, and the mean value for the 48 squares was used to calculate the mean root dry matter per plant. The root dry matter (kg ha^{-1}) was calculated by multiplying the root mean dry matter per plant by the plant density (number of plants per hectare).

At maturity, 10 plants were randomly harvested from each

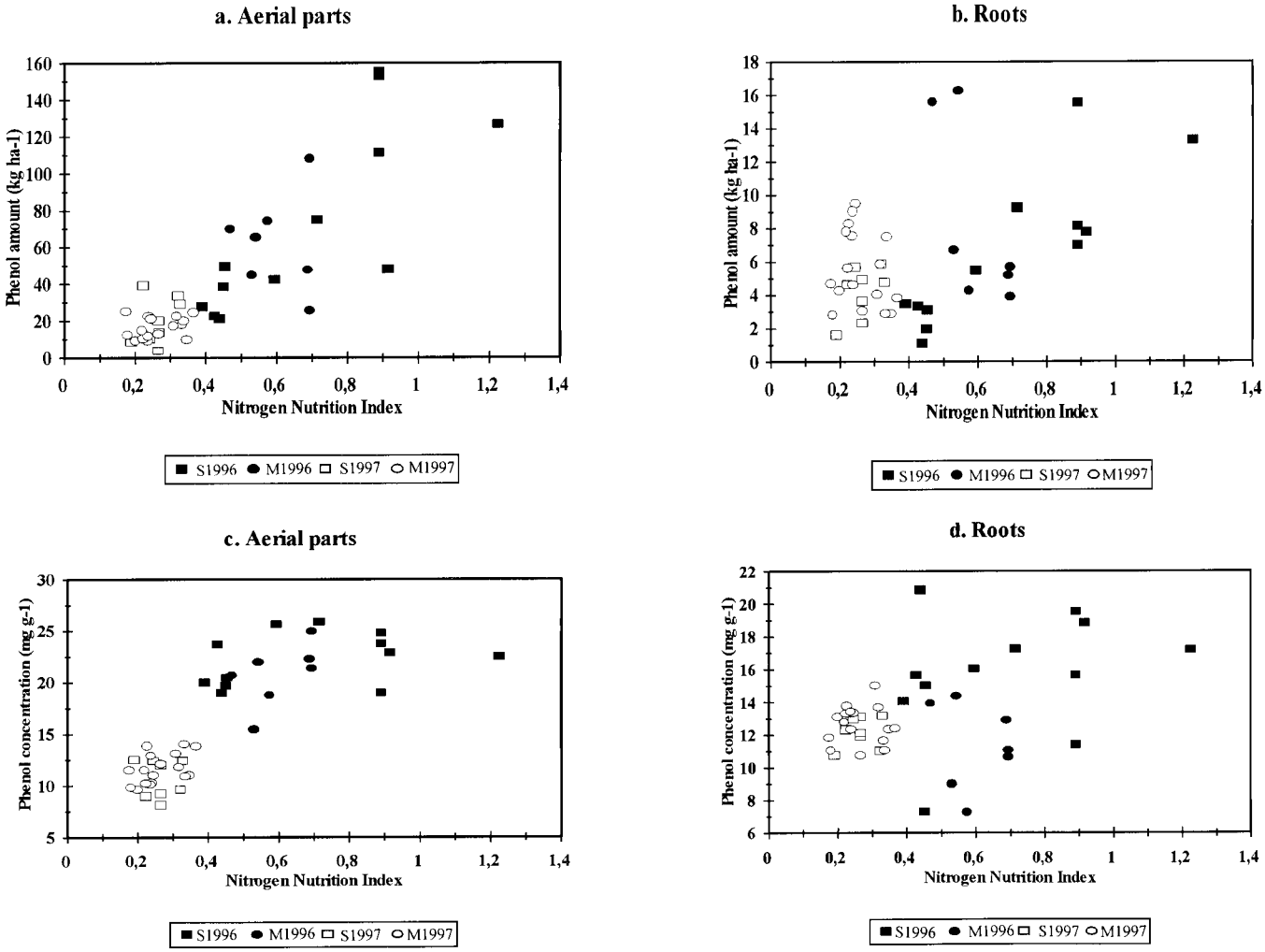

Fig. 2. Relationship between (*a*) the total phenol amount in the aerial parts and the NNI and (*b*) the total phenol amount in the roots and the NNI and between (*c*) the total phenol conc. in the aerial parts and the NNI and (*d*) the total phenol conc. in the roots and the NNI. S1996 and S1997: Fields at the Sagnanème site in 1996 and 1997; M1996 and M1997: Fields at the Médina site in 1996 and 1997.

of the five yield subplots in each field, and the panicles were removed and air-dried for 30 d. The grain was removed and weighed, and the yield was calculated for each subplot. The grain yield of each field (kg ha^{-1}) was estimated as the mean of the five subplot yields.

The work of Plénet (1995) and Lemaire et al. (1996) was used to assess the N nutrition. These authors established a reference curve (after Greenwood et al., 1990; Lemaire et al., 1990) for maize (*Zea mays* L.) and sorghum, establishing the relationship between aerial dry matter (Mg ha^{-1} DW) and the normal N concentration in aerial dry matter (Nc), expressed as %DW using the equation

$$Nc = 3.4DW^{-0.37}$$

The reference value (Nc) derived from the reference dilution curve and the percentage of N in the sorghum crop aerial dry matter (Nt) was then used to give the NNI

$$NNI = Nt/Nc$$

According to Lemaire and Gastal (1997) and Plénet and Cruz (1997), the lower the NNI, the poorer the N nutrition. We could therefore compare the N nutritional status of crops that differed considerably in growth.

Phenolic Analysis of the Plants

Dried, ground-up roots or aerial parts (500 mg) were extracted twice (100 and 75 ml) by refluxing with 70% (v/v) boiling ethanol (C_2H_5OH) for 20 min. The extracts were pooled and evaporated under vacuum. The residues were dissolved in a standard volume of hot distilled water, and aliquots were taken for the determination of total phenol, using the Folin–Ciocalteu reagent and gallic acid as a standard. The results are expressed as milligrams of gallic acid equivalents.

Statistical Analysis

Pearson correlation coefficients were calculated between phenol concentrations and the NNI, the phenolic amount and plant dry matter, the phenolic concentration and plant dry matter, and the phenolic amount and grain yield.

RESULTS

The total phenol amount in the aerial parts varied among fields from each data set and among sets. In 1996, it ranged from 22 to 156 kg ha^{-1} in Sagnanème

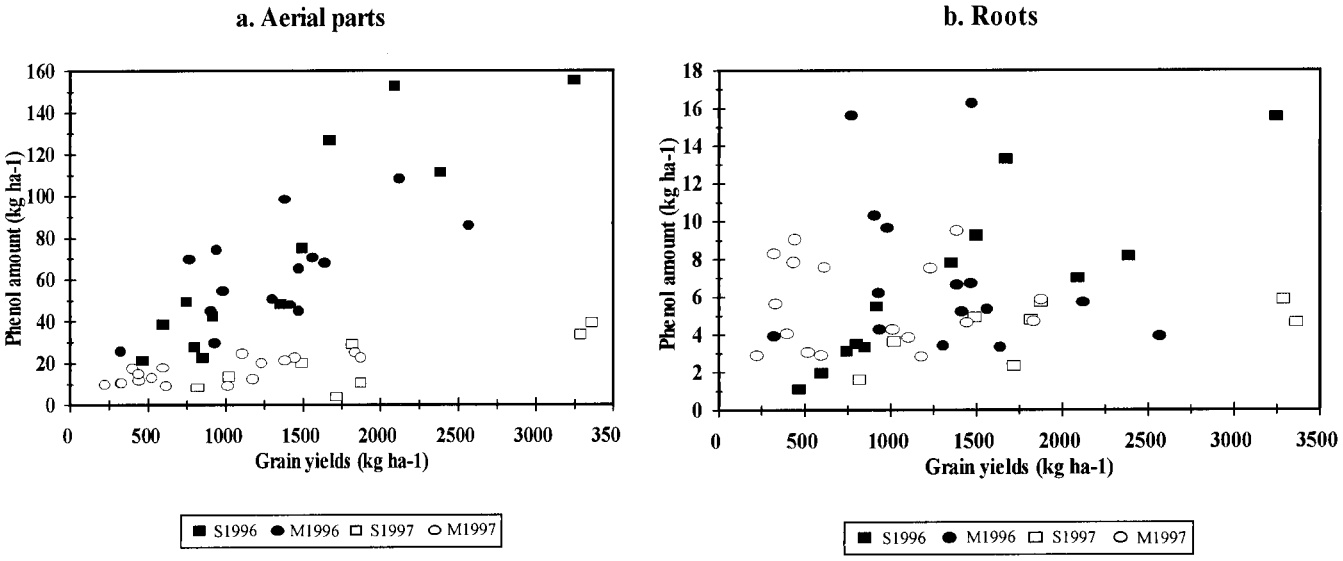

Fig. 3. Relationship between the grain yield and total phenol amount in (*a*) the aerial parts and (*b*) roots. S1996 and S1997: Fields at the Sagnanème site in 1996 and 1997; M1996 and M1997: Fields at the Médina site in 1996 and 1997.

and from 26 to 98 kg ha^{-1} in Médina. In 1997, it ranged from 4 to 39 kg ha^{-1} in Sagnanème and from 9 to 25 kg ha^{-1} in Médina. There were also wide differences in the phenol amount in the roots. In 1996, it ranged from 1 to 15 kg ha^{-1} in Sagnanème and from 3 to 16 kg ha^{-1} in Médina. In 1997, it ranged from 2 to 6 kg ha^{-1} in Sagnanème and from 3 to 9 kg ha^{-1} in Médina. For both the aerial parts and roots, the total phenol amount was closely correlated with the dry matter (Fig. 1a, 1b). The coefficients of correlation for the dry matter and total phenols were $r = 0.95$ ($P < 0.001$) in the aerial parts and $r = 0.91$ ($P < 0.001$) in the roots. When the years and sites were considered separately, the coefficient of correlation was always higher than 0.9 ($P < 0.001$) for the roots and 0.8 ($P < 0.001$) for the aerial parts, except for Sagnanème in 1997 ($r = 0.65$, $P = 0.08$). In contrast, the total phenol amount was linked less with the phenol concentration ($r = 0.40$, $P = 0.004$ for phenol conc. and total phenols in the aerial parts and $r = 0.21$ $P = 0.142$ in the roots) (Fig. 1c, 1d).

The accumulation of phenols in the aerial parts and roots of sorghum plants in the fields seemed to be positively correlated with the NNI ($r = 0.85$, $P < 0.001$ and $r = 0.46$, $P = 0.002$ for the aerial parts and roots, respectively) (Fig. 2a, 2b). A similar but less marked trend was found for the phenol concentrations ($r = 0.81$, $P < 0.001$ for the aerial parts and $r = 0.38$, $P = 0.011$ for the roots) (Fig. 2c, 2d). These correlations disappeared when the years and sites were considered separately, except for the correlation between the total phenol amount and NNI at Sagnanème in 1996 ($r = 0.80$, $P = 0.0018$ for aerial parts and $r = 0.85$, $P < 0.001$ for the roots).

For each set of data, a close correlation was found between the total phenol amount in the aerial parts and grain yield: r ranged from 0.66 to 0.90 ($P < 0.02$ for each data set) (Fig. 3a). A positive correlation between the phenols in the roots and grain yield was also observed at the Sagnanème site in both 1996 and 1997 ($r = 0.86$ and 0.81, $P < 0.001$ and 0.002 in 1996 and 1997, respectively) but not in Médina ($P > 0.07$) (Fig. 3b).

DISCUSSION

Studies of allelopathy in sorghum have focussed on the phenol concentrations (Guenzi and McCalla, 1966; Woodhead and Bernays, 1978; Woodhead and Cooper-Driver, 1979; Burgos-Leon et al., 1980; Cherney et al., 1991; Einhellig et al., 1993; Ben-Hammouda et al., 1995), rather than the total phenol amount.

In the four data sets that we studied, an increase in the dry matter amount of the roots and aerial parts was associated with an increase in the total phenolic amount. The range of dry matter was greater (by a factor of up to 20 in the aerial parts and up to 25 in the roots) than the range of phenol concentrations (a factor of about 3 for both the aerial parts and roots). Thus, the total phenolic amount of sorghum tissues was linked more with the dry matter production than with the phenol concentration. This finding is crucial in assessing the potential release of phenolic allelochemicals by a sorghum crop. This was not expected because biotic or abiotic constraints were assumed to enhance the phenol concentration and decrease dry matter; thus the result on the total phenol amount was unclear. Using the concentration of phenols to assess the potential release in a cropping system is of limited value unless the plant dry matter is determinated, as was recently emphasized by Koricheva (1999).

The growth conditions varied considerably among years, sites, and the individual fields in each data set, as shown by the wide range of grain yield. Nitrogen nutrition also varied among fields within each data set for 1996, as shown by the values of the NNI for this year. However, this index only reflects the N nutrition status of the crop and does not provide information on other limiting factors, such as water supply. We nevertheless observed a positive correlation between the total

phenol amount and the NNI across sites and years. This correlation was basically due to the existence of a wide difference between the 2 yr for each of the measured parameters. When the sites and years were considered separately, differences between data sets emerged and the correlation remained only for Sagnanème in 1996. The differences between data sets may be attributed to differences in growth conditions between the sites and years. This is especially true of the total rainfall during the growing season, which varied from 396 mm in Médina in 1996 to 932 mm in Sagnanème in 1997. The water stress that occurred in 1996 might explain the increased phenolic production because a moderate water deficit is believed to stimulate secondary metabolites synthesis (Horner, 1990). If the magnitude of the water deficit on the phenolic production is larger than that of N nutrition, it could partly explain the positive correlation across sites and years between the concentration of total phenols (especially in the aerial parts) and the NNI when the C/N balance hypothesis postulates a decrease in the C-based secondary metabolites as N fertilization increases. For instance, Dustin and Cooper-Driver (1992) found a decrease in the total phenol concentration in the leaves of hay-scented fern (*Dennstaedtia punctilobula*) when the foliage N concentrations increased. The discrepancy between our data and those of others may be due to: (i) the difference in the species used because most of the earlier studies were obtained on tree species (see the review of Koricheva et al., 1998) and (ii) the difference between the NNI and N concentration, the latter being less effective as an indicator of N deficiency. However, the correlation for the fields as a whole was no longer observed within the four separate data sets. Therefore, our results that are related to the relationship between N nutrition and phenol accumulation might have a limited value. Such a correlation must be checked under controlled conditions, in which N nutrition is the only variable.

Finally, we observed a correlation between the total phenol amount and grain yield of the sorghum crops. This was true of all of the data sets for the aerial parts, and it is probably due to the influence of dry matter accumulation on both the grain yield and total phenol amount. This correlation was found for the roots only in two data sets (Sagnanème, 1996, 1997). The between-set differences could be attributed to differences in the shoot/root ratio (DW basis). The fields with high yields apparently took advantage of better growth conditions and had crops with high total phenol amounts. It could be useful to design agronomic strategies that allow subsequent crops to escape the impact of the phenols that are released, rather than trying to decrease the phenol production because grain yield and phenol production by sorghum (CE_{145-66}) are linked.

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Rust-Enhanced Allelopathy of Perennial Ryegrass against White Clover

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ABSTRACT

Perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) are important pasture components in the higher rainfall areas of southeastern Australia. Crown rust (*Puccinia coronata* Corda f.sp. *lolii* Brown) is the most serious ryegrass pathogen in these areas. In a preliminary investigation, rust reduced ryegrass biomass by 56%. Yet, interference from rusted ryegrass suppressed the yield of neighboring clover plants more than interference from healthy ryegrass. The role of allelopathy in this relationship was investigated in a greenhouse study using two bioassays. Soil previously growing rusted ryegrass suppressed clover biomass by 36% compared with soil previously growing healthy ryegrass. Similarly, leachate from soil surrounding rusted ryegrass suppressed clover biomass by 27% compared with that from healthy ryegrass. This is the first demonstration that a pathogen may influence allelopathy between plants and that rust may enhance ryegrass allelopathy against clover. Possible implications of this in pasture ecology and the evolution of mutualism are discussed.

Perennial ryegrass and white clover predominate improved pastures in the higher rainfall areas of southeastern Australia. Their growth in mixtures without added nitrogen results in greater herbage yields than otherwise can be achieved economically (Menchaca and Connolly, 1990). Crown rust is the most serious ryegrass fungal pathogen in these areas (Eagling and Clark, 1993), with epidemics regularly occurring between spring and autumn. A preliminary study demonstrated that rust accelerates senescence and reduces ryegrass yield by 56% (Mattner, 1998). Despite this, in ryegrass and clover mixtures, interference from rusted ryegrass suppressed clover biomass by up to 47% compared with interference from healthy ryegrass. This suppression did not result from a direct effect of crown rust on clover, because rust-inoculated and non-inoculated clover monocultures yielded the same, which was expected since clover is a nonhost of crown rust. Similarly, clover suppression is not explained by rust increasing ryegrass competitiveness because, if this were so, the reduction in clover yield would be greatest at high densities where resources are most limited. Instead, clover suppression was greatest at low densities, where competition for resources was minimal. Ryegrass allelopathy is well documented, particularly against clovers and medics (Gussin and Lynch, 1981; Takahashi et al., 1988, 1991, 1993; Quigley et al., 1990; Chung and Miller, 1995). For these reasons, we investigated the hypothesis that rusting increases ryegrass allelopathic ability.

Both Rice (1984) and Einhellig (1995) hypothesized that pathogens enhance their host's allelopathic ability. Evidence supporting this hypothesis occurs in at least two forms. Firstly, pathogens stimulate phytoalexin (antimicrobial compounds) production by their hosts (Smith, 1996), which can belong to similar chemical groups and are synthesized via the same biochemical

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