Abstract A chemosystematic study of leaf phenolics was carried out on 464 seedlings from 34 populations of seven subspecies of *Acacia nilotica* from its native range and 5 populations in Queensland where it has been introduced.

These seven subspecies of *A. nilotica* can be grouped into three main chemotypes based on the HPLC chromatogram fingerprints of their soluble leaf phenolics. The first chemotype is represented by *A. n. leiocarpa*; the second by a subgroup of *A. n. kraussiana*; and the third consists of the remaining *A. n. kraussiana, A. n. indica, A. n. cupressiformis, A. n. subalata, A. n. adstringens* and *A. n. tomentosa*. The third chemotype can be split further into three subtypes, one containing *A. n. indica* and *A. n. cupressiformis*, one containing remaining *A. n. kraussiana, A. n. subalata* and *A. n. adstringens* and another consisting of *A. n. tomentosa*.

In Queensland, the weedy *A. nilotica* is confirmed to be of the subspecies *indica*, in accordance with existing morphological identification. No evidence of hybridization of the Queensland material with other subspecies was observed in the HPLC chromatograms.

INTRODUCTION

*Acacia nilotica* (L.) Willd. ex Del., known in Australia as prickly acacia, infests over $7 \times 10^6$ ha of the Mitchell grass downs in Queensland. The economic impact of prickly acacia on the beef grazing industry could be as much as A$8-9$ m annually due to reduced production and control costs (Mackey 1997). The environmental impact *A. nilotica* is difficult to quantify but is believed to be high.

Biological control is the preferable alternative to large-scale chemical treatment or mechanical clearing. Surveys of the insect fauna of *A. n. indica* (Benth.) Brenan in Pakistan and *A. n. leiocarpa* Brenan and *A. n. subalata* (Vatke) Brenan in Kenya (Marohasy 1995) have been conducted, resulting in the release of three agents into Australia. However, only one has successfully established and is having a minimal effect on the spread of the weed. The search for new agents is therefore essential and may be facilitated if the Australian weedy material can be better matched to *A. nilotica* in its native range, and insect faunal surveys conducted accordingly. The taxon in Queensland has been identified as *A. n. indica*. However, variation in pod morphology, hearsay-reports of introductions from Africa, and at least one reported case of possible hybrid sterility, suggest that introductions may have been made from other parts of the native range (Mackey 1997) of *A. nilotica*.

*A. nilotica* is a polymorphic species and is considered to be represented by nine subspecies (Brenan 1983). The species is widely distributed in subtropical and tropical Africa, from Egypt and Mauritania to South Africa and in the Indian subcontinent. Brenan (1983) considered the subspecies of *A. nilotica* to be morphologically and ecologically distinct. Subspecies have been differentiated mainly on the shape, size and degree of pubescence of the pods. The degree of pubescence of the young branchlets, the habit of the tree, and the shape of the crown are also important identifying characteristics.

Host selection behaviour of insects for either oviposition or herbivory may be determined by factors such as the physical properties of the plant, i.e. shape, size and colour, surface texture, and chemical constituents. There is some evidence (Bernays and Chapman 1994) that secondary metabolites, such as phenolics and flavonoids, can act as either deterrents or phagostimulants depending on the insect species, the secondary metabolite and its concentration. These metabolites can thus help define or limit the insect’s host range.

Two dimensional thin layer chromatography or paper chromatography have traditionally been used for the identification of flavonoids and other phenolics in plant material. More recently high pressure liquid chromatography (HPLC) has been employed (Markham 1989, van Sumere 1989). Tamma et al. (1985) illustrated
the HPLC separation and quantification of flavonoids and coumarins in subspecies of *Artemisia*, revealing the potential for chemotaxonomic application.

The primary aim of this investigation was to ascertain if the Australian weedy *A. nilotica* is or is not of hybrid origin. If it were determined not to be of hybrid origin then which subspecies is it most like, if it were a hybrid then which subspecies were involved. The home range or ranges of these subspecies could then be surveyed for insect and pathogenic fauna, with the intention of introducing potential biological agents for the control of *A. nilotica* in Australia. Another objective was to determine the relatedness of the various subspecies, to assist in prioritising the order in which the remaining subspecies could be surveyed in the ongoing worldwide search for biological control agents.

**MATERIALS AND METHODS**

*A. nilotica* seeds were obtained from seven of the nine recognised subspecies by field collection from 34 populations in their native ranges in Africa, Indian subcontinent, and from five weedy populations in Queensland, Australia. The two subspecies absent from this study are *A. n. nilotica*, which occurs from Ethiopia through northern tropical Africa to Senegal, and *A. n. hemispherica*, which is reported to occur only near Karachi, Pakistan. After scarification and soaking in warm water for 16 hrs, approximately 50 seeds were germinated, and 10 seedlings grown in climate controlled glasshouses (temperature cycle 26°C day, 23°C night, 50% humidity, natural lighting) to standard age (six months) before harvest. All leaves (leaflets plus petiole) were removed, freeze-dried and stored at –25°C until analysed. The samples were extracted by soaking 0.5 g of the milled material in 5 mL of ethanol-water (70:30, v/v) for 16 hrs after an initial 30 minutes of ultrasonication. Each sample was thoroughly mixed and filtered through a 0.45 µm filter. A 20 mL aliquot was injected onto the HPLC system.

Peak selection was based on the presence of a particular compound in at least one of the samples with a peak area count of greater than 1000. The peak area data for each particular constituent was normalised relative to the largest area shown in any of the samples for that constituent, to give a score from 0 to 100. This procedure was followed so that all components would have equal contribution, ensuring that the major constituents in any sample would not skew the analysis.

Principal components analysis (PCA) was conducted on the normalised peak data. The discriminant analysis was conducted as either complete, where all peak variables were included, or as a forward or backwards stepwise iterative process, where peaks were added or removed depending on preset $F$-statistic values.

**RESULTS**

Twenty-two prominent peaks were detected and used in the statistical analysis. Varimax rotation of the PCA loadings enhanced the association of the peaks with the first two factors. The loadings of the principal components showed that the first three components accounted for 43.6% of the total variation of within the samples.

The first two principal components were plotted against each other (Figure 1). This plot discriminated the Kenyan *A. n. leiocarpa* and “Group 1” *A. n. kraussiana* from all the other material. The *A. n. indica* from all sources and *A. n. cupressiformis* samples were not differentiated from each other. The *A. n. kraussiana* samples were split into two sub-clusters corresponding to the two groups based on the presence or absence of peak. One of these clusters, corresponding to “Group 2” *A. n. kraussiana*, was not well differentiated from the *A. n. indica* and *A. n. cupressiformis* cluster. Clusters for *A. n. subalata*, *A. n. tomentosa* and *A. n. adstringens* overlay the *A. n. indica* and *A. n. cupressiformis* and “Group 2” *A. n. kraussiana* clusters. The weedy Queensland *A. nilotica* clustered with *A. n. indica*. The plots of the first v third and second v third principal component scores did not give any improved differentiation.

Discriminant analysis was used to further differentiate the confused clusters of the Queensland material from the *A. n. indica* and *A. n. cupressiformis* cluster and the clusters for *A. n. subalata*, *A. n. tomentosa* and *A. n. adstringens*. A subset of the normalised peak area data set, including only these samples was examined. As with PCA, the Queensland material, *A. n. indica* and *A. n. cupressiformis* remain tightly clustered (Figure 2). However, the clusters of *A. n. tomentosa*, *A. n. subalata* and *A. n. adstringens* were differentiated from each other and from the *A. n. indica* and *A. n. cupressiformis* cluster. The “Group 2” *A. n. kraussiana* overlaps with each of the *A. n. subalata* and *A. n. adstringens* clusters.

Finally, a cluster tree for the subspecies, based on the group means of the normalised peak area scores, was produced (Figure 3).
The seven subspecies of *A. nilotica* examined in this study can be grouped into three major chemotypes based on the multivariate similarity of their HPLC phenolics/flavonoid chromatograms. *A. n. leiocarpa* belongs to the first chemotype, “Group 1” *A. n. kraussiana* in the second, and the remaining subspecies in the third. I will refer to these as the “leiocarpa”, “kraussiana” and “indica” chemotypes respectively.

The chromatograms of the Queensland material fall within the general “indica” chemotype. Close examination of the chromatograms of the Queensland samples did not reveal additional major or minor peaks not also found in the *A. n. indica* from its native range (i.e. no hybrid specific or another subspecies specific compounds). The various analyses of the data suggest that the Queensland material is nothing other than “pure” *A. n. indica*.

The *A. nilotica* subspecies examined have been divided into two morphological groups wherein the mature pods are either “necklace like, narrowly and regularly constricted between seeds”, or with “margins straight or crenate” (Fagg and Greaves 1990). The first group is represented by the African *A. n. tomentosa* and *A. n. nilotica*, and the Indian *A. n. indica* and *A. n. cupressiformis*. The second group consists of the African *A. n. leiocarpa*, *A. n. subalata*, *A. n. kraussiana*, *A. n. adstringens* and the Indian *A. n. hemispherica*.
It is also worth noting that seedlings of the various subspecies exhibited some broad differences in their early growth characteristics, although exact measurements were not recorded. One form (Form A) consisting of A. n. indica, A. n. cupressiformis, A. n. adstringens and A. n. tomentosa, was erect and smooth barked, while the other form (seedling Form B) (A. n. kraussiana, A. n. leiocarpa and A. n. subalata) was spreading, with rough, fissured bark. The stems of the Form B seedlings were also thicker at the base (ground level) and the seedlings of very obviously lesser height at a given age than those of Form A. After the leaves were harvested, the plants were cut back to a single 20 cm long stem and all side branches removed. Plants of Form A then continued to produce one or two erect dominant leaders while plants of Form B produced multiple lateral or prostrate branches.

Divisions on pod shape and seedling form do not directly correlate to the chemotypes, but some association of “necklace” pods with seedling Form A and the “indica” chemotype does exist.

Mandal and Ennos (1995) have indicated that A. n. kraussiana is a predominantly outcrossing subspecies, in contrast to A. n. leiocarpa, which has shown a significant level of self pollination (Mandal et al. 1994). The phenolic patterns or HPLC fingerprints of A. n. leiocarpa are more homogeneous than those of A. n. kraussiana, which is consistent with the proposed breeding system for these subspecies.

It is suggested that the Queensland A. nilotica is indistinguishable from A. n. indica and A. n. cupressiformis from the Indian subcontinent. A. n. subalata, A. n. adstringens and “Group 2” A. n. kraussiana are near to the Queensland material in their leaf phenolic composition. The African subspecies A. n. tomentosa is next most similar in composition. The “Group 1” A. n. kraussiana and A. n. leiocarpa are the least like the Queensland material.

If it is assumed that these phenolic patterns correlate with host range defining stimulants and deterrents, it might be concluded that insects utilising leaves that closely matched the Queensland weedy material would be the most suitable for assessment as biocontrol agents. On this basis, I suggest the following order for examination of the subspecies for potential biocontrol agents: A. n. indica, then in order A. n. cupressiformis, (A. n. subalata, “Group 2” A. n. kraussiana, A. n. adstringens), A. n. tomentosa, “Group 1” A. n. kraussiana and finally A. n. leiocarpa.

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