agriculture,
forestry & fisheries

Department:
Agriculture, forestry & fisheries
REPUBLIC OF SOUTH AFRICA

GUIDELINES FOR THE PREPARATION, EXECUTION AND ASSESSMENT OF AGROCHEMICAL FIELD TRIALS IN SUGARCANE IN SOUTH AFRICA

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1. INTRODUCTION

The purpose of this document is to provide users with guidelines for conducting agrochemical field trials in the sugar industry. It must be used in conjunction with the guidelines already published by the office of the registrar. This document supersedes all other sugarcane related guidelines, and is aimed in particular at new molecules and generics. Where there is a variation, it will be specified. Note that all agrochemical formulations and agrochemical tank mixtures, including adjuvants etc. require registration under Act 36/1947. It is recommended that the South African Sugarcane Research Institute (SASRI) be kept informed of the progress of field trials if results are to be used to support product registration in sugarcane. The SASRI Director Dr C Baker, can be contacted at Carolyn.Baker@sugar.org.za and the SASRI Operations Manager, Mrs K Redshaw at Kerry.Redshaw@sugar.org.za. This document is effective as from the 1st April 2018.

1.1. Trial requirements

1.1.1. Data from three efficacy trials and five residue trials are required for registration of an agricultural remedy. Residue trials must be according to the residues guidance document published by the office of the Registrar in 2016. For herbicides, data from a minimum of three field phytotoxicity trials are also required. For agrochemicals that must be applied aerially, one additional trial for efficacy and residues using aerial spraying is required over and above the ground application trials.

1.1.2. Trials should be located in all three bioclimatic zones of sugarcane (see Appendix 1). If the pest is only found in one or two bioclimatic zones, then trials in any single zone should be located at least 100km apart. If this is not possible, trials should be conducted over two seasons. More details can be obtained from the general data requirements guidelines available on the DAFF website (http://www.daff.gov.za/daffweb3/Branches/Agricultural-Production-Health-Food Safety/Agriculture-Inputs-Control).

1.1.3. Specified plot dimensions are the minimum plot size required. The number and length of rows can be increased but not reduced. Please note that all treatments including the untreated control should be in the same field.

1.1.4. Plots should be arranged in a randomized complete block design. If this design is not possible due to product application, this should be specified upfront. Plot sizes vary depending on the agrochemical tested and will be discussed within the relevant section.

1.1.5. For efficacy and phytotoxicity trials, treatments should consist of an untreated control, the candidate product at 1X and 2X the recommended rate and the industry standard. For residue trials, only the candidate product at the highest recommended rate and an untreated control are required. Each treatment in efficacy and phytotoxicity trials should be replicated at least four times. To increase the chance of obtaining statistically significant results however, six replicates are recommended. Replication of treatments in residue trials is not required.

1.1.6. Where heights and counts are required, the heights of 20 randomly selected stalks should be taken along one nett row and all the stalks in that row should be counted. Stalk height should be measured from ground level to the top visible dewlap (see Appendix 2).

1.1.7. Yield assessments (cane and sucrose) are required for molecules belonging to categories i and ii, as defined in the general data requirements guidelines available on the DAFF website (http://www.daff.gov.za/daffweb3/Branches/Agricultural-Production-Health-Food Safety/Agriculture-Inputs-Control). Yield and quality data is not required for categories iii and iv if there is no change in GAP.

1.1.8. Cane yield is obtained by weighing all the stalks in the nett rows and expressing these weights in tons cane per hectare. Sucrose yield is determined by taking 12 stalks from the nett rows and sending to a qualified lab for analysis.

All data should be analysed statistically to identify any significant differences between treatments.

1.2. Other key considerations

1.2.1. For soil applied pesticides, soil samples from each site should be analysed beforehand. The selection of appropriate soil type, conditions and chemistry is important to minimize confounding factors. Soil analysis should be done in laboratories that are Agri-Lasa affiliated or ISO17025/GLP accredited.

1.2.2. The varieties used in pesticide trials. These should be susceptible to the target pest/disease. More than one variety should be used across trials where possible.

1.2.3. Topography in relation to trial design, layout and treatment.

1.2.4. Incidence of target pest/disease/weed or crop nutrient deficiency/excess. This information may influence the timing of product application or help interpret trial findings.

1.2.5. Choice of application methods, equipment and spray conditions. (e.g. water pH, nozzle selection, equipment calibration, wind speed).

1.2.6. Water quality. If municipal water is not used to prepare agrochemical mixes, a water sample must be submitted to a reputable laboratory for analysis. This is to ensure that the pH of the water does not affect the efficacy of the agrochemical.

1.2.7. Selection of appropriate methods to correctly assess product efficacy.

1.2.8. Standard methods for operations such as soil and leaf sampling in sugarcane should be followed.

1.2.9. When products are foliar applied, unsprayed guard rows are recommended between plots to minimise drift effects.

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2. HERBICIDES

2.1. Efficacy trials

2.1.1. Trials should be established to cover as wide a range as possible of the weed species which are encountered in the South African sugar industry.

2.1.2. Plot size is influenced by weed distribution and presence or absence of the cane crop. However, plots should be large enough to ensure that there is no spray interference from the adjacent plots. The nett plot on which ratings are done should exclude end effects (the outer portions of the plot). A plot size is therefore required of at least 2 nett interrows x 5 m nett length plus one sugarcane guard row on each side (gross plot size 5 rows x 6m).

2.1.3. The untreated control should be randomised within the trial.

2.1.4. The weed control rating system used should be indicated and clearly explained.

2.1.5. Application dates, dilution rates, soil conditions, climatic conditions prior to and after application, crop growth stage, weed control ratings and any other relevant information such as application technique and equipment should be recorded.

2.1.6. The spectrum of weeds controlled and important weed species that are not controlled must be indicated.

2.2. Field phytotoxicity trials

2.2.1. In an efficacy trial, crop tolerance or phytotoxicity can also be evaluated, provided the cane crop is short, and efficacy evaluations **are not confounded** by shading/water depletion etc. by tall weeds or the crop.

2.2.2. Where sampling for residues is required; each plot should consist of at least six rows x 6m. The outer two guard rows (rows 1 and 6) are left unsprayed. Growth and yield measurements are taken from the nett rows 3 and 4. Sampling for residues is taken from rows 2 and 5. When this sampling is done, it should be done in such a way that no gaps should be left in the rows, i.e. one stalk every few metres.

Where sampling for residues is not required; each plot should consist of at least four rows x 6m. The outer two guard rows (rows 1 and 4) are left unsprayed. Growth and yield measurements are taken from rows 2 and 3.

2.2.3. Treatments should include an unsprayed control and a control that has been hand-weeded.

2.2.4. For **pre-emergence weed control**, of the three trials required, one should be on plant cane in a sandy soil, sprayed soon after planting. Another should be on plant cane in a clay soil, **sprayed after the cane crop has already emerged.**

2.2.4 For **post emergent weed control**, separate trials should be conducted for plant and ratooncane. Trials should be conducted in the following sequence (Fig. 1), with treatments being sprayed onto the foliage once the cane has fully tillered.

a) Sandy soils: Conduct plant and ratoon cane trials on sandy soils. If no phytotoxicity is observed compared with industry standards, the recommendation is that the chemical is safe for use on both sandy and clay soils on PLANT cane or RATOON cane. If phytotoxicity is observed in sandy soils when compared with industry standards, then conduct plant and ratoon cane trials in clay soils.

b) Clay soils: If no phytotoxicity is observed compared with industry standards, the recommendation is that the chemical is safe for clay soils but not for sandy soils. If phytotoxicity is observed in the PLANT cane when compared with industry standards, then the chemical is unsafe for use on PLANT cane. If phytotoxicity is observed in the RATOON cane when compared with industry standards, then the chemical is unsafe for use on cane.

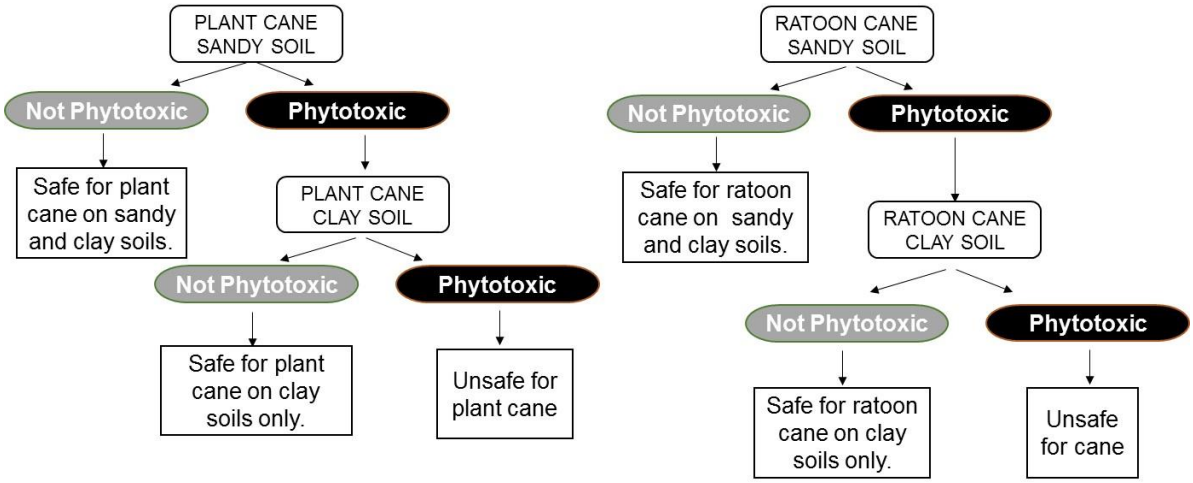

Figure 1. Schematic diagram of requirements for post emergence herbicide phytotoxicity trials.

For yield assessments. Refer to point 1.1.7. Data required for product assessment includes soil analysis and climatic conditions at spraying, as well as periodic rating for symptoms such as scorch/chlorosis/necrosis and stunting. Growth measurements and stalk population counts should be carried at least twice after application, and also at harvest (when harvest is required). At harvest, yield (tons cane/ha) and a full analysis of cane quality is also required for category (i) and (ii) registrations. This is taken at the normal 12-18 months crop age, depending on the growing region. For category (iii) products (new formulations of products that are already registered on sugarcane), trials should be taken through to yield when the effects of the new formulation on sucrose and yield are not known.

In the case of category (iv) products (generic equivalents of products that are already registered on sugarcane), provided that the product is being compared with the registered equivalent, the trial should be carried out to yield only if there is phytotoxicity observed in the qualitative assessments that have been carried out at regular intervals after treatment.

2.3. Phytotoxicity trials in trays or pots

Pot/tray trials are useful for rain-fastness and safener trials since they make use of single budded setts under controlled irrigation conditions, with measurements taken to compare different treatments. Refer to point 1.1.1., which stipulates that a minimum of three phytotoxicity trials should also be conducted under field conditions.

2.3.1. Treatments shall consist of:

2.3.1.1. Control (hand-weeded – no herbicide applied).

2.3.1.2. Current commonly registered herbicide and candidate herbicide at the recommended dosages.

2.3.1.3. Current commonly registered herbicide and candidate herbicide at double the recommended dosages.

2.3.2. At least three sugarcane varieties should be used in pot trials. These include NCo376 as the standard, as well as one herbicide sensitive (N14, N25, N31), and one tolerant variety (N27).

2.3.3. Two soil types (sand and sandy clay loam) should be used, preferably with six replications in pot trials, to account for variability.

2.3.4. Treatments should, in the case of pre-emergence herbicides, be applied to the soil soon after planting or in the case of a post-crop emergence herbicide, directly over the cane foliage when this has reached the stage of 3 to 5 leaves unfurled per shoot, or as applicable to the particular herbicide.

2.3.5. Stalk height (ground to top visible dewlap) or number of unfurled leaves should be taken before treatment in pots. A periodic rating of early damage symptoms that might be temporary e.g. scorch or chlorosis is useful before harvesting stalks from pots. Stalk height measurements and mass of above-ground parts should be recorded approximately 12 weeks after planting in the case of a pre-crop emergence treatment and about six weeks after application of a post-emergence treatment. This data should be used to assess the phytotoxicity of the treatment.

3. GROWTH REGULATORS APPLIED TO LEAF CANOPY (E.G. CHEMICAL RIPENERS)

3.1. Plot size

Each plot should consist of six 8 - 10m long rows. Rows 1 and 6 are regarded as guard rows and must not be treated. Destructive measurements can be taken on rows 2 and 5. Rows 3

and 4 are regarded as nett rows and non-destructive (growth) measurements are taken on these rows. Full details of the time and method(s) of application must be recorded.

3.2. Ripener Efficacy trials

3.2.1. The activity of the chemical must be reflected by statistically verified ($P < 0.05$) increases of sucrose content (%) and sucrose yield (t/ha) above that of untreated cane (control). Demonstrating increases in Brix is not sufficient. Ideally the effects on whole stalk juice purity (%) and fibre content (%) should also be determined.

3.2.2. Sequential sampling from rows 2 and 5 in each plot, to demonstrate the efficacy on sucrose content and cane mass, should be such that the optimal interval between spraying and harvesting can be established to maximise the benefits in terms of sucrose yields (see 4.1.4). Sampling of end-effects (outer 1 m on each side of cane rows) should be avoided, due to the risk of non-uniform product application.

3.2.3. The efficacy of the chemical being tested must be compared with at least one of the commercially available registered products and untreated cane (control). A rate of twice the intended registered rate should be included specifically in trials which will be continued in the following ratoon to assess any negative residual effects on ratoon regrowth.

3.2.4. For sequential sampling purposes a minimum of 12 stalks from each plot should be collected for sucrose content analysis and cane mass assessment. Green tops should be removed at the natural breaking point at the top of the stalk. All remaining green and dead leaves must be removed.

3.2.5. At harvest, the effect of the chemical on cane yield (t/ha) must be assessed to enable accurate estimation of sucrose yield (t/ha). The two centre cane rows (rows 3 and 4) in each plot are harvested and weighed for estimation of cane yield (t/ha). Harvesting of end-effects (outer 1 m on each side of cane rows) should be avoided. In order to explain any possible effects of the chemical on cane yield the total number of millable stalks in one of these cane rows (excluding end effects), and the height of at least 16 randomly selected stalks from ground level to the top visible dewlap leaf, should be determined prior to harvest.

3.2.6. All visual symptoms related to the chemical's action on the plant should be recorded.

3.2.7. Appropriate agronomic practices should be followed throughout the course of each trial and these recorded.

3.3. Residual effects on crop regrowth

3.3.1. The effect of the chemical, at the intended rate of registration and at double the rate, on the emergence and growth of the crop following that to which it is applied, should be monitored in all trials. Assessment of growth should be done by means of the measurements (stalk height and number) detailed under 3.2.5.

3.3.2. Should negative residual activity be detected at any stage of growth, the cane and sucrose yields of individual plots at harvest must be assessed (see 3.2.5 for method).

3.4. Application methods

3.4.1. The test chemical should be applied to cane rows 2 – 5 in each plot while cane rows 1 and 6 act as unsprayed guard rows. All spray mixtures should be applied over the cane canopy by a constant-pressure (for example CO_2-pressurised) knapsack with a hand held boom fitted with appropriate nozzles spaced according to the trial row spacing. The spray mixtures should be delivered in a water volume of at least 50 l/ha.

3.4.2. Aerial spray trials should be applied such that untreated control strips are left (randomised and replicated), or covered plots are used to provide a source of samples from untreated cane.

4. NEMATICIDES

4.1. Pre-trial requirements

Trial sites should be analysed for soil physical properties (%clay, silt and sand). Because nematicide responses vary with respect to clay percentage, a variety of clay percentages should be tested. Also, be aware that nematicides (and many agrochemicals) are unstable at high pH's, so identify any liming practices at proposed trial sites as these may pose a risk to product efficacy. A soil and root sample must also be sent to a reputable lab for nematode analyses to determine the nematode community present in the soil. To ensure adequate responses it is preferable to use sites where the nematode community comprises either singly, or combinations of *Meloidogyne, Xiphinema* or *Paratrichodorus*. These are the species that are most pathogenic to sugarcane.

4.2. Plot size

Each plot should consist of five 10 m rows, all treated. The two outer rows are regarded as guard rows and destructive measurements are usually taken on these rows. The three middle rows are regarded as nett rows and growth measurements are usually taken on these rows. Where the product is sprayed, two extra untreated guard rows are recommended to minimise the effect of spray drift on results.

4.3. Timing of application

In plant cane, the product should be applied in the furrow at planting. For safety reasons, ensure that this is the last operation before closing the furrow. In ratoon crops, treatments should be applied over the cane row within four weeks of harvesting of the previous crop in summer (in winter application can be delayed slightly). Apply granular nematicides using a correctly calibrated wheelbarrow applicator and liquid nematicides using a calibrated knapsack or CO_2-regulated spray apparatus. If product specifications differ from current recommendations, this must be clearly stated.

4.4. Efficacy assessment

4.4.1. To ensure that any reduction in nematode numbers is detected, frequent sampling should be conducted during the early stages of the trial. Monthly sampling is recommended for at least 3 months after treatment application. However to obtain statistically significant results, monthly sampling for six months is recommended.

4.4.2. Samples should be taken from the guard rows (the outer treated rows of a plot - row 1 or 5) by placing a spade right next to the growing cane and digging out both roots and soil to a depth of approximately 20-30cm. Ensure that the top drier layer of soil is removed before placing the remaining soil into a sample bag. Cut roots off the sampled stool and place into the same bag. Ten samples, (one every 2m), should be taken along the two guard rows and placed into 1 bag. To ensure it fits into 1 bag, ten small samples should be taken. Ensure that samples are processed as soon as possible and prevent them from drying out by tightly sealing/enclosing the sample bags.

5. INSECTICIDES

5.1. Plot size

5.1.1. **Eldana**: Each plot should consist of ten 15m long rows all treated. The outer four rows are used for destructive measurements while the middle six rows are regarded as nett rows which are used for growth and yield purposes.

5.1.2. **Thrips and yellow aphid**: Each plot should consist of five 10 m rows, all treated. The two outer rows are regarded as guard rows and destructive measurements are usually taken on these rows. The three middle rows are regarded as nett rows. Non-destructive and growth measurements are usually taken on these rows.

5.1.3. Should aerial application be required a trial design appropriate to this application method is necessary. Such a design can comprise divisions of whole fields either as strip plots comprising the swath width of the aircraft (plus a suitable guard area) or easily demarcated areas of fields that can be used for “treated and untreated” comparisons.

5.2. Treatment application

Products must be applied in a manner that will reflect probable commercial application procedures. The selected method may comprise knapsacks, modified knapsacks or aircraft. Ensure that the appropriate nozzle is used on a correctly calibrated knapsack. Details of application methods, products used, rates and formulations need to be defined prior to trial treatment (e.g. types and number of nozzles, system design and spray target).

5.3. Efficacy assessments

Periodic assessment of pest numbers is required over the life of the trial. Also, crop yield estimates are required at harvest for the required categories. Choice of harvest date may be important (i.e. seasonal or carry-over crops). Treatment impact on crop yield needs to be assessed based on plot yields (tons cane) and estimates of sucrose yield (tons sucrose).

5.4. Sampling of trials

Sampling frequency and pattern will be determined by the pest species involved. The aim of sampling is to attempt to reliably assess pest numbers/damage without bias and the sampling pattern selected must reflect this. Guidance is given for three major sugarcane pests: eldana, thrips and yellow sugarcane aphid.

5.4.1. **Eldana**

5.4.1.1. At least three estimates of eldana larval numbers and damage should be made, over the life of the trial, the last and most important estimate being at harvest. Each estimate will comprise a 25 stalk sample from each plot. Stalks will be assessed for damage and the values “% internodes bored, % stalks bored and larvae per 25 stalks will be recorded. When required, a 12 stalk sample is collected from each nett plot for further analysis (e.g. quality) and nett plots are weighed at harvest.

5.4.1.2. A set sampling pattern cannot be given as plot size may vary. However, based on what was stated above, six stalks are randomly collected from each of the outer four plot rows (one additional stalk should be taken at random from one of the outer rows to make up 25 in total). Samplers must walk along each row, plucking a stalk every two metres along the row, providing six stalks per row. The initial sampling position must be altered for each survey to ensure the same position in the row is not sampled twice.

5.4.2. **Thrips and yellow aphid**

5.4.2.1. For treatments applied in the furrow at planting (September/March), at least two estimates of thrips and aphid numbers are required typically between 4 and 12 weeks after germination. In the case of thrips, trials planted mid-October to mid-December receive maximum thrip pressure and assessments of thrips numbers and damage in both January and February has been shown to optimally discriminate between treatments. For thrips, at least five sugarcane spindle samples are required per plot. These can be taken in a diagonal pattern across the length of the plot – one spindle per nett row. The pattern can be varied for subsequent samples. Care must be taken to cut only the spindle leaves and not cut the growing point of the tiller. Samples should be placed in plastic bags (per plot) and counted within a day of sampling unless stored in a deep freeze. Counting comprises the water extraction of thrips from plant material and counts made of adult and juvenile stages of the pest. For aphids, counts should be done on the lowest green leaf. Select five random samples within the plot and count all the aphids on the leaf. In the absence of thrips and aphids counts, an appropriate damage rating scale can be used. When required, a 12 stalk sample is

collected from each nett plot for further analysis (e.g. quality) and nett plots are weighed at harvest.

5.4.2.2. For foliar application trials in ratoon cane, maximum discriminating power between treatments for thrips is usually obtained when cane is ratooned November/December. Generally, aphid infestations are less predictable.

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6. FUNGICIDES

6.1. Trial requirements

The seasons and regions selected should favour the development of the disease being targeted. Fungicides can be applied as sett treatments (for diseases such as smut and pineapple sett rot) or foliar sprays (for diseases such as rust).

6.2. Plot size

Each plot should consist of five 10 m rows. The two outer rows are regarded as guard rows and are left untreated. The three middle rows are regarded as nett rows and growth measurements are usually taken on these rows.

6.3. Sett treatments

6.3.1. **Product application**

Setts must be soaked in the product solution for a specified time (usually 10 minutes) before planting. The untreated controls are soaked in water for the same period. Setts may be sprayed in the furrow using a knapsack applicator. This is not as effective as dipping the setts but can be included **as an additional treatment**. This should not replace the sett dip treatment.

6.3.2. **Assessments**

For systemic diseases such as smut, total stalks and the number of infected stalks in the three nett rows are counted six weeks after planting depending on the growing conditions, and again three to four months later. If symptoms of phytotoxicity are evident e.g. leaf scorching and die-back, high resolution photographs should be taken for assessment. The initial tiller/stalk counts will give an indication of any effect of the product on germination. The heights (ground to top visible dewlap) of seven stalks / nett row for each plot should be measured three to four months after treatment. Ideally, heights and counts should also be taken just before harvest.

6.4. Foliar treatments

6.4.1. **Product application**

It may be necessary to apply a product more than once and the design will need to take this into account. Foliar fungicides should be applied with a calibrated knapsack or CO_2-regulated spray apparatus fitted with an appropriate nozzle. Good foliar coverage with minimal drift has been achieved with a blue fan jet nozzle APE110. It is advisable to add a mist control product to the fungicide mix to reduce drift.

6.4.2. **Assessments / sample collection**

6.4.2.1. The heights (ground to top visible dewlap) of seven stalks per nett row and counts (total tillers/nett row) should be taken before treatment. A further assessment is required three to four months after the final treatment. A final assessment of height and stalk number is recommended just before harvest.

6.4.2.2. The severity of a foliar disease such as rust is assessed by collecting five leaves from each nett row immediately before and two weeks after each treatment. Disease severity should be assessed using a software package such as APS ASSESS 2.0. Phytotoxicity assessments (e.g. leaf scorching and die back) should be conducted at the same time. An appropriate rating scale can also be used. An overall assessment of disease severity in the treated lines is also recommended.

The following trial protocol is not a requirement for registration of a nutritional product but is supplied as a recommendation for how nutritional trials can be conducted in sugarcane.

7. NUTRITIONAL PRODUCTS

7.1. Crop nutrition products

Sugarcane crop nutritional products come in three basic categories:

7.1.1. Group 1: Inorganic fertilizers such as superphosphate, MAP, LAN and potash, as well as blends and compounds. Amendments such as limes and gypsum also fall in this category.

7.1.2. Group 2: Liquid and solid formulations of macro and micro-nutrients, with various 'growth-enhancers' such as enzymes and organic compounds (typically humic and fulvic acids, and sea-weed extracts).

7.1.3. Group 3: Liquid formulations of macro and micro-nutrients. Certain of the micronutrients may be in organic or chelated form.

7.2. Testing product efficacy

7.2.1. Testing of single-nutrient inorganic (Group 1 above) fertilisers such as superphosphate and potash is relatively simple. A typical procedure would involve the following:

7.2.2. Identification of the 'indicator' crop (in this case sugarcane).

7.2.3. Selection of a site (soil) which is deficient in the nutrient in question.

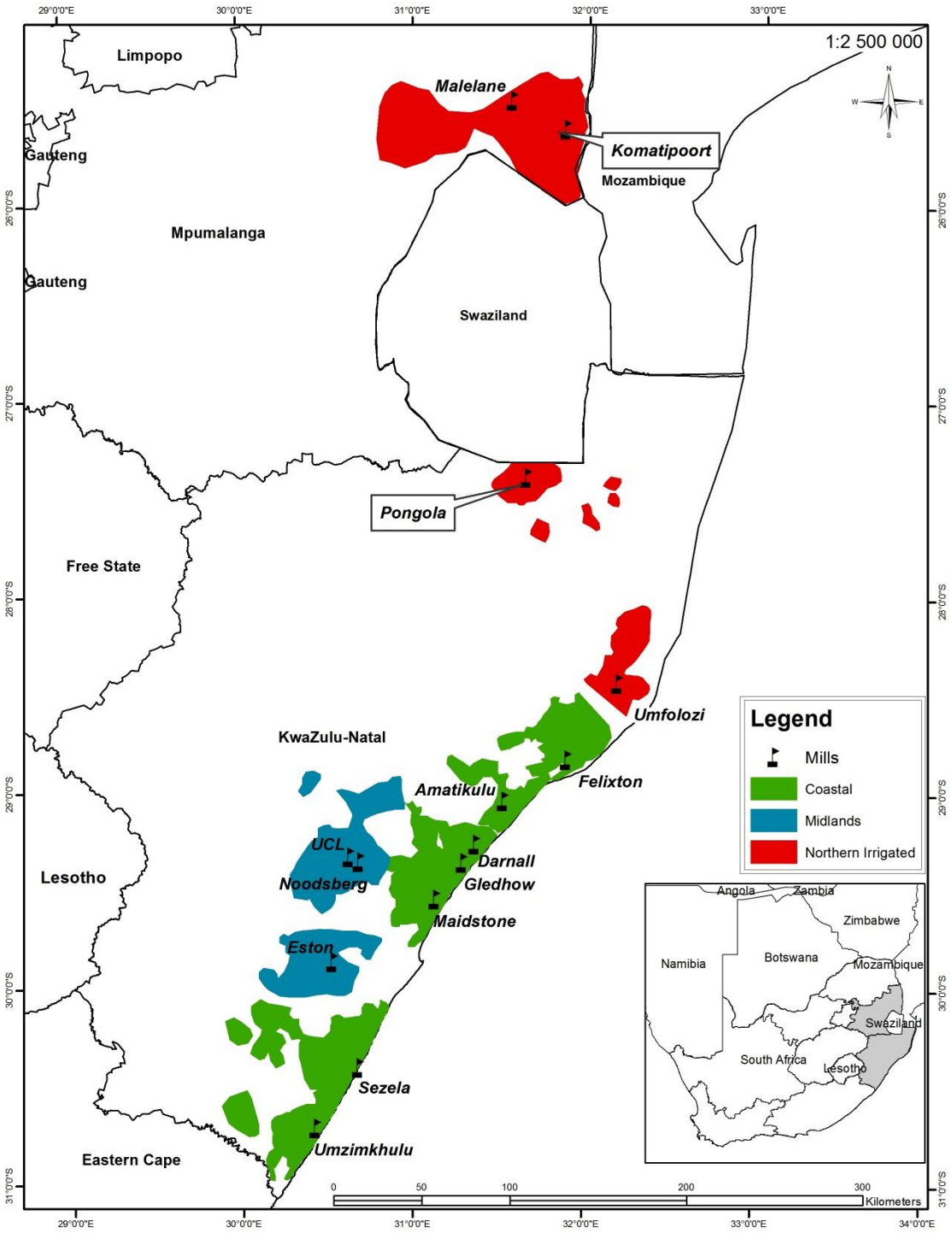
7.2.4. Applying treatments which supply incremental rates of the test nutrient based on soil type and the nutrient being tested. Treatments should always include a control (zero application) of the product under investigation.

7.2.5. Ensure the supply of adequate levels of non-test nutrients to the trial site (i.e. if an N fertiliser carrier was being tested, adequate supplies of P, K, Ca, Mg, S, Zn etc). Such requirements can be determined from the analysis of pre-trial soil samples.

7.2.6. Assessments should include soil tests to determine product effects on soil properties, plant (leaf) analysis to gauge nutrient uptake, and yield at harvest, both tons cane/ha and tons sucrose/ha estimates (the latter obtained from a qualified facility).

7.2.7. For testing the efficacy of the products listed under 7.1.2 and 7.1.3 above, no standard procedures exist. However, the trial designs can follow the protocol for category 1 products noted above. The products could be applied at incremental rates, with a zero control included, and crop measurements undertaken as detailed above. However, where responses are shown to products containing more than one nutrient or compound, difficulties do arise in determining the factor(s) responsible for these and it will be an important part of the trial protocol to precisely identify the factors involved in measured responses.

APPENDIX 1: Bioclimatic zones for sugarcane production in South Africa



Zone	Average cutting cycle	Total long term mean Annual Rainfall (mm)	Monthly long term mean Min. Temp range (^{0}C)	Monthly long term mean Max. Temp range (^{0}C)	Average long term mean A pan Evap. (mm/d)
Coastal	18	936	11 – 20	23 – 28	3.2
Midlands	24	790	7 – 15	20 – 26	3.7
Northern	12	712	9 - 20	24 - 31	4.2

APPENDIX 2: Sugarcane height measurement to top visible dewlap (TVD)