GUIDELINES FOR TRIALS PRIOR TO REGISTRATION OF CHEMICAL CONTROL AGENTS FOR USE ON TOBACCO: 2000

Released by the Registrar: Act 36 of 1947 and drafted by the Institute for Industrial Crops in collaboration with AVCASA.

CONTENTS

INTRODUCTION

1. The prime requisite for registration is that the product must be suitable and sufficiently effective for the purpose for which it is intended and that it is not contrary to the interest of the public that it be registered [Section 3(2) Act 36/1947].

2. Experimentation with the object to obtain registration of an agricultural Plant protection agent (PPA) must be discussed in advance with the technical advisors of Act 36/1947, and with the Institute for Industrial Crops (IIC) of the Agricultural Research Council (ARC).

COMMENTS

INTRODUCTION POINT 2:

AVCASA:

According to the Agricultural Remedies Registration Procedure Policy Document March 1998, "regulations require that prior to the commencement of any trials the Registrar must be informed in writing of the intention to conduct such trials in order that he may inspect their performance".

Regulations therefore do not require that trials must be in discussed with any institution except the Registrar. Development programs for registration are discussed with the technical advisors of the registrar of Act 36/1947 and in most cases with an appropriate expert, usually within the ARC. It should therefore be a recommendation, but not a requirement.

INDUSTRY:

We see the draft document as a combined effort from AVCASA, the registrar, tobacco industry and IIC and it is therefore regrettable that members of AVCASA expect a negative attitude from the institute. The institute acts as an advisor for the Registrar as well as the industry for the use of PPA's. The name of the document is "Guidelines for trials prior to registration....." and therefore does not imply absolute requirements. The IIC cannot make any rules and in fact do not want to. We do not think the intention of the inclusion of IIC in the sentence was to make it a requirement, but rather an opportunity to sort out expected problems. Perhaps we could include: "It is suggested that the manufacturer discuss the protocol of the evaluation with the IIC before commencing trials". This could only be for the benefit of the manufacturer.

3. It is recommended that the IIC is regularly informed of the progress of all experiments prior to submission of a product for registration.

4. As the end product is used for human consumption, residue trials must be done according to the requirements of the Registrar (Act 36/1947) (Section 6).

5. Experimental work should be done on a sound biometrical basis in order that the results can be analysed statistically.

COMMENTS

INTRODUCTION POINT 5:

AVCASA:

We agree in principle with this statement, but there are certain exceptions where it is impractical and where alternative techniques exist. Should not alternative methods, for example Dr Drinkwater's strip ("strook") method for pre-plant incorporated insecticides against black maize beetle, be considered for other

products too?

INDUSTRY:

Point 5 is exactly according to "Pesticide regulations in South Africa" (pg 53) of which you sent us a copy. We did not make this rule.

6. Experimental data in support of claims made for new products (new active ingredients) should be derived from experiments over at least **two** and preferably **three** seasons. Research should, where applicable, be done in different climatic conditions and on different soil types in areas of the RSA where the PPA will be applied. The types of tobacco for which registration is intended, should be used in the trials.

7. At least **three** trials should be done, but the actual number of trials will be determined during the initial discussions with the IIC and the respective advisor.

Comments:

INTRODUCTION POINT 7

AVCASA

Proposed change:

The actual number of trials will be determined during initial and subsequent discussions with the technical adviser of Act 36/1947.

What about generic registrations?

INDUSTRY:

Act 36 specifies three trials. If "discussions with IIC" is a problem we will remove it. Regarding generic compounds the "Agricultural remedies registration procedure policy document" of March 1997exempts a number of compounds which are listed. However, under the same heading it also states that "If new formulations are developed efficacy and/or phytotoxicity data may be required". The tobacco industry has had problems in the past with certain generic products being phytotoxic which indicates that generic products are not always identical to the original product.

8. Details must be furnished about the type of equipment used for the application of the PPA. This should include the spray nozzles used as well as the application pressure and the volume of diluted chemical applied per hectare.

COMMENTS

INTRODUCTION POINT 8.

AVCASA:
Proposed change:
This should include the spray nozzles used, as well as the application pressure, volume of diluted chemical applied per hectare, droplet coverage etc, where applicable.

INDUSTRY:
We agree with the proposed change.

9. In the presentation of the results (see Section 5) on the effect of PPA's, all adverse effects of the chemicals, for example phytotoxicity, must be recorded and discussed.

10. The effect of the PPA upon the taste and quality of the tobacco must be determined by smoke tests (Section 7).

COMMENTS

INTRODUCTION POINT 10:

AVCASA:
Proposed change:
The effect of the PPA upon the taste and quality of the tobacco must be determined by smoke tests where applicable, after consultation with the technical adviser of Act 36/1947 (Section 7)"
(See also comments on Section 7.)

INDUSTRY:
This is a requirement of the tobacco industry and the reason why should be self explanatory. Our market is at stake.

11. All trials for registration of chemical PPA's should be done by suitably qualified persons.

COMMENTS

INTRODUCTION POINT 11:

AVCASA:
Please define "suitably qualified persons".

INDUSTRY:

Someone who can do the job.

12. Data from other countries may be submitted **only in support** of the application for registration.

SECTION 1: BACTERICIDES AND FUNGICIDES

LOCALITY

Do the trials in areas where tobacco is grown commercially and where the PPA will be applied.

Where two trials are done in one area during a season they should be on separate farms to ensure that the respective trials are done under conditions that are different for the specific area.

Where possible, trials should be on different soil types.

TRIAL LAY-OUT

1. A minimum of 4 replications should be used per treatment and at least 3 treatments resulting in a minimum of 18 degrees of freedom per trial in a randomised experimental plan.

COMMENTS

SECTION 1 TRIAL LAY-OUT – 'MINIMUM OF 4 REPLICATIONS'

AVCASA

The minimum number of replications needed for statistical analysis should be determined by the number of treatments.

INDUSTRY

We agree with the comment. We could add "Where possible, use four replications for more reliable results".

2. Minimum plot size to be 20 m^2. Plots should preferably consist of 4 rows with the two middle rows serving as data rows.

3. A trial should include:

3.1 The expected dosage rate and half and double the expected dosage rate. Adjustment of rates can be made in subsequent experiments.

COMMENTS

SECTION 1 TRIAL LAY-OUT POINT 3.1.

AVCASA

Why require half dosage? Half dosage is seldom included in trials. One to several dosages (within a known efficacy range) are normally included in the first trial and then streamlined during further development. What company, for example, will test half dosage of a generic product.?

INDUSTRY:

Agree with comment. 3.1 could read: "One to several dosages could be included in the first trial and then reduced during further development".

3.2 A comparable standard PPA or -programme chosen in consultation with the advisers.

3.3 An untreated control.

EVALUATION

1. Black shank (*Phytophthora nicotianae* var. *nicotianae*)

Percentage healthy plants are to be determined three weekly up to at least 15 wk after transplanting in an infested field. The first count should be made 1 wk after transplanting.

COMMENTS

SECTION 1, EVALUATION, POINT 1:

AVCASA:
We consider this requirement of a minimum infection level of 50% too high for practical purposes.

INDUSTRY:
Fields with a 100% black shank infestation are plentiful. Please define "too high for practical purposes".

2. Rhizoctonia leafspot, shot hole [*Thanatephorus cucumeris* (*Rhizoctonia solani*)

Trials with this pathogen would probably have to be done in seed trays. This can be under conditions of natural or artificial infection. The number of plants with sore shin or damping off or the percentage leaf area with shot hole or leaf spot should be determined.

The number of plants that are healthy (suitable for transplanting) at the end of the period in the seed tray is important and must be recorded.

COMMENTS

SECTION 1, EVALUATION, POINT 2:

AVCASA:

Can these trials with this pathogen only be done in seed tray trials and will this be acceptable for registration purposes?

INDUSTRY:

Hierdie voorstel was bedoel vir waar die siekte as 'n bedding of saailaaisiekte voorkom. Dis tog seker voor die hand liggend dat daar nie saailaaiproewe gedoen gaan word as die siekte slegs op volwasse tabak in die land voorkom nie.

GENERAL COMMENT

INDUSTRY:

A few diseases and pests were included under Section 1 and 2 with possibilities or ideas how trials could be done and results taken. This was not meant as a regulation, but seems to have been interpreted as such - surely the commentators know there are more than two diseases in tobacco. We suggest that everything said about specific diseases and pests be deleted from the document.

SECTION 2: INSECTICIDES AND MITICIDES

LOCALITY

Do the trials in areas where tobacco is grown commercially and where the PPA will be applied.

Where two trials are done in one area during a season they should be on separate farms to ensure that the respective trials are done under conditions that are different for the specific area.

Where possible trials should be on different soil types. Seed- and soil treatments should be tested in low pH and high pH soils.

COMMENTS

SECTION 2: LOCALITY:

AVCASA:

It is usually known beforehand if the efficacy of a specific product is pH related or not. This statement is only applicable if the working of the product is pH sensitive. There are also many other factors that may influence efficacy for example, low and high clay percentages, etc.

Since products are applied commercially under highly variable conditions, they are also tested under conditions varying as widely as possible. It is however impossible to specify and make allowance for every possible condition that may, or may not, occur.

INDUSTRY:

Propose: "Where it can be done trials should be on different soil types and under conditions varying as widely as possible".

The infestation potential of the insect/mite concerned, should be adequate in the area where the experiment is to be done.

When pheromone and other monitor systems are evaluated, such trials should be discussed with the appropriate advisor beforehand.

TRIAL LAY-OUT

1. A minimum of 4 replications should be used per treatment and at least three treatments resulting in a minimum of 18 degrees of freedom per trial in a randomised experimental plan. Pre-treatment data, where applicable, is important in the planning of a well laid out experiment..

COMMENTS

SECTION 2 TRIAL LAY-OUT POINT 1. – 'MINIMUM OF 4 REPLICATIONS'

AVCASA

The minimum number of replications needed for statistical analysis should be determined by the number of treatments.

INDUSTRY

We agree with the comment. We could add "Where possible, use four replications for more reliable results".

2. Minimum plot size to be 20 m^2. Plots should preferably consist of 4 rows with the two middle rows serving as data rows.
3. An experiment should include:

3.1 Expected dosage rate and half and double the expected dosage rate.

Adjustment of rates can be made in subsequent experiments.

COMMENTS

SECTION 2, TRIAL LAY OUT POINT 3.1.

AVCASA

Why require half dosage? Half dosage is seldom included in trials. One to several dosages (within a known efficacy range) are normally included in the first trial and then streamlined during further development. What company, for example, will test half dosage of a generic product.?

INDUSTRY:

Agree with comment. 3.1 could read: "One to several dosages could be included in the first trial and then reduced during further development".

3.2 A comparable standard PPA or -programme chosen in consultation with advisors.

3.3 An untreated control.

EVALUATION

1. Boll worm (*Helicoverpa armigera*)

Count the insects before applying treatments and preferably at 1-, 4-, 7- and 14 d after treatment. Where follow -up treatments are applied, counts could be made weekly.

Examine 10-12 plants per plot if the infestation level is high. If the infestation level is low, examine all plants.

All instars should be counted and could be recorded in the following size classes: 5-10 mm (L1 & L2), 10-20 mm (L3), >20 mm (L4 & L5).

Where control is obtained by a number of sprays, possible damage to leaves and the growing-points could also be determined.

2. Aphids (*Myzus spp.*)

Count the insects before application of the insecticide and at 1-, 4-, 7- and 14 d thereafter. Where follow-up treatments are applied, counts could be made weekly.

Examine 12 plants per plot and 3 leaves per plant (1 leaf at top of plant and 2 leaves approximately one third from the top of the plant).

3. Thrips (*Thrips tabaci*)

Count the insects before treatment and preferably at 1-, 4-, 7- and 14 d thereafter. Where follow-up treatments are applied, counts could be made weekly. Count all sizes.

Count the insects on 12 plants per plot or count the number of plants with and without damage. Counting procedure can be as for aphids.

Damage estimates (counts) could be considered if a compound has a long residual effect or when it is applied a number of times resulting in the appearance of new growth. If this is the case, examine at least 3 grow points and 4 sub-apical leaves from 12 plants per plot for insect damage.

4. Mites

Please refer to procedures listed for aphids. However, mites can only colonise the plants when trichomes no longer exude their sticky substances. It will therefore be required to examine older leaves.

COMMENTS

SECTION 2, EVALUATION POINTS 1 - 4:

AVCASA:

Years of research experience has probably demonstrated that this methods are suitable to evaluate the specific pests, but it is necessary the only acceptable methods. The proposed evaluation dates for bollworm, aphids and thrips for example are impractical for field trials, where localities are hundred of kilometres away. Evaluations at weekly intervals from the start are more practical. Furthermore when dealing with most pests, like aphids and mites, making use of severity classes and indexes are far more practical and less time consuming than exact counts. Depending on the plot size, it will also be impractical to examine all the pants for bollworm. Why should 12 plants per plot be examined for aphids? What is wrong with ten plants, which is far more practical, when percentages are to be calculated? Registration trials are seldom conducted by, or close by, research institutions and guidelines and evaluation methods should therefore be scientifically acceptable, but also practical under field conditions.

Proposed change:

The methods heading should be changed to "EXAMPLES OF RECOMMENDED EVALUATION TECHNIQUES FOR SPECIFIC PESTS" and more practical evaluation methods, times and guidelines should be included.

5. White fly

Please refer to procedures listed for aphids for trials with whitefly.

GENERAL COMMENT

INDUSTRY:

A few diseases and pests were included under Section 1 and 2 with possibilities or ideas how trials could be done and results taken. This was not meant as a regulation, but seems to have been interpreted as such - surely the commentators know there are more than two diseases in tobacco. We suggest that everything said about specific diseases and pests be deleted from the document.

SECTION 3: NEMATICIDES

LOCALITY

Both pre- and post planting treatments should be done in areas where specific nematodes species cause problems.

Trials should preferably be done on soil types varying in clay content and must include a light sandy soil as well as a heavier clay soil. Phytotoxicity of a nematicide may differ in soil types with varying clay and organic material content.

TRIAL LAY-OUT

With the exception of the nematicides, all treatments and untreated control must receive the same treatment regarding soil preparation, irrigation, disease-, insect- and weed control. **(No systemic pesticides should be used during the maintenance period.)**

COMMENTS

SECTION 3 TRIAL LAY-OUT

…No systemic pesticides should be used during the maintenance period.

AVCASA:

Proposed change

No systemic pesticides which have efficacy against nematodes should be …

INDUSTRY

Agree with proposed change some systemic pesticides have no effect on namotodes.

1. Enough replications should be used per treatment in order to comply with the

statistical requirements for a minimum of 18 degrees of freedom per trial.

COMMENTS

SECTION 3 TRIAL LAY-OUT POINT 1. – 'MINIMUM OF 4 REPLICATIONS'

AVCASA

The minimum number of replications needed for statistical analysis should be determined by the number of treatments.

INDUSTRY

We agree with the comment. We could add "Where possible, use four replications for more reliable results".

2. Nematode counts from representative soil samples must be done by a qualified nematologist directly before the trial begins. This is required to confirm the presence and numbers of plant parasitic nematodes.

COMMENTS

SECTION 3 TRIAL LAY-OUT POINT 2

AVCASA

"Nematode count from representative soil samples must be done by a qualified nematologist directly before the trial begins. This is required to confirm the presence and numbers of plant parasitic nematodes."

In most cases the nematode count data does not justify the high costs involved. There is a tendency evolving within research institutions (outside and within the

ARC) that nematode counts are considered less and less important in nematicide efficacy trials. Some institutions even regard it as optional.

The questions that should be asked are, what is our goal, when doing nematode counts and are we achieving it?

INDUSTRY:

Statistically proven results must show that the PPA is effective against the specific pest.

GENERAL COMMENT

AVCASA:

Let us take the pre-trial soil count and later soil counts as examples:

At the beginning of the growing season of annual crops, plant parasitic nematodes are often not in an active stage, or occurs below the root zone where soil samples are taken, or only occurs concentrated in small areas of a field. Detailed nematode counts (per plot) in soil at this stage (or for that matter at later stages) usually results in totally useless and confusing data, due to very low soil counts at the beginning (before the susceptible crop is planted) and large variation in soil counts. Even root counts often do not provide a clear picture of the actual situation due to large variation.

Under controlled conditions where artificial inoculations are done, or where a susceptible crop, like beans, are planted to activate and build up nematode populations before a nematode trial commences, nematode counts may be worthwhile. Field trials (for registration purposes) are however not conducted under ideal conditions and therefore the current emphasis placed on nematode counts should be re-considered, especially taking into account the high costs involved in counts. Several nematicides, at the dosages applied, does not

necessary directly kill the nematodes. Control may also be achieved by disorientation of nematodes, damaging the cuticle, enhancing natural enemies, enhancing plant growth and resistance, etc.

At the end of the day it is increased yield and quality of the crop that are the important parameters. Farmers are not satisfied with reduced nematode counts after applying a nematicide, they are only satisfied with increased crop yield and/or quality. The use of other parameters to establish the presence of nematodes, like root gall indexes with root knot nematodes for example, can also substitute counts.

It is long overdue for us to take into count the high cost involved in doing nematode counts and ask ourselves: Would not our end purpose be served equally well by substituting nematodes counts with other suitable parameters? Or by only establishing the presence and numbers of nematodes only with one nematode root count (after planting) in control plots? Or doing only one nematode root count in all plots, for example six or twelve weeks after planting? Please comment.

INDUSTRY:

Nematodes have a devastating effect on tobacco and one must be certain that you are controlling the pest. Nematode numbers should be determined at the end of the previous crop when they do occur in the root zone.

There are other ecto-parasites like the stubby root- and stunt nematodes that also cause problems in tobacco and for them soil counts are necessary. Most companies do their evaluations on the root knot nematode only, but then they should specify this on their label. Then they cannot say that their substance controls nematodes – and that without any counts. Different nematode species react differently to nematicides, e.g. one specific nematicide gave excellent

control of a lesion nematode while another had no effect on it whatsoever.

It is so that certain nematicides only disorientate the nematodes, but hungry nematodes quickly perish and then you won't count them anyway.

The farmer will be satisfied with a higher yield, yes, but does your label specify a higher yield or effectivity as a nematicide, and what happens to the follow-up crop if the nematodes are not controlled?

3. Treatments should include:
3.1 The expected dosage
3.2 Half the expected dosage and one and a half to double the expected dosage

COMMENTS

SECTION 3 TRIAL LAY-OUT POINT 3.2.

AVCASA
Why require half dosage? Half dosage is seldom included in trials. One to several dosages (within a known efficacy range) are normally included in the first trial and then streamlined during further development. What company, for example, will test half dosage of a generic product.?

INDUSTRY:

Agree with comment. 3.2 could read: "One to several dosages could be included in the first trial and then reduced during further development".

3.3 A comparable standard, chosen in consultation with advisors

3.4 An untreated control

FIELD TRIALS

4. The minimum plot size should be ∀ 40 m^2. Each plot must preferably consist of 4 rows with the two middle rows (∀20 m^2) serving as data rows.

5. Data acquisition should include the following aspects:

5.1 Nematode counts from representative soil samples from each plot taken:

5.1.1 Prior to application of chemicals and prior to planting;

5.1.2 Six weeks after planting;

5.1.3 Twelve weeks after planting.

5.2 Nematode counts from representative root samples collected from each plot:

5.2.1 Six weeks after planting;

5.2.2 Twelve weeks after planting.

COMMENTS

SECTION 3 FIELD TRIALS POINT 5.1 AND 5.2

AVCASA

"Nematode count from representative soil samples must be done by a qualified nematologist directly before the trial begins. This is required to confirm the presence and numbers of plant parasitic nematodes."

In most cases the nematode count data does not justify the high costs involved. There is a tendency evolving within research institutions (outside and within the ARC) that nematode counts are considered less and less important in nematicide efficacy trials. Some institutions even regard it as optional.

The questions that should be asked are, what is our goal, when doing

nematode counts and are we achieving it?

INDUSTRY:

Statistically proven results must show that the PPA is effective against the specific pest.

GENERAL COMMENT

AVCASA:

Let us take the pre-trial soil count and later soil counts as examples:

At the beginning of the growing season of annual crops, plant parasitic nematodes are often not in an active stage, or occurs below the root zone where soil samples are taken, or only occurs concentrated in small areas of a field. Detailed nematode counts (per plot) in soil at this stage (or for that matter at later stages) usually results in totally useless and confusing data, due to very low soil counts at the beginning (before the susceptible crop is planted) and large variation in soil counts. Even root counts often do not provide a clear picture of the actual situation due to large variation.

Under controlled conditions where artificial inoculations are done, or where a susceptible crop, like beans, are planted to activate and build up nematode populations before a nematode trial commences, nematode counts may be worthwhile. Field trials (for registration purposes) are however not conducted under ideal conditions and therefore the current emphasis placed on nematode counts should be re-considered, especially taking into account the high costs involved in counts. Several nematicides, at the dosages applied, does not necessary directly kill the nematodes. Control may also be achieved by disorientation of nematodes, damaging the cuticle, enhancing natural enemies, enhancing plant growth and resistance, etc.

At the end of the day it is increased yield and quality of the crop that are the important parameters. Farmers are not satisfied with reduced nematode counts after applying a nematicide, they are only satisfied with increased crop yield and/or quality. The use of other parameters to establish the presence of nematodes, like root gall indexes with root knot nematodes for example, can also substitute counts.

It is long overdue for us to take into count the high cost involved in doing nematode counts and ask ourselves: Would not our end purpose be served equally well by substituting nematodes counts with other suitable parameters? Or by only establishing the presence and numbers of nematodes only with one nematode root count (after planting) in control plots? Or doing only one nematode root count in all plots, for example six or twelve weeks after planting? Please comment.

INDUSTRY:
Nematodes have a devastating effect on tobacco and one must be certain that you are controlling the pest. Nematode numbers should be determined at the end of the previous crop when they do occur in the root zone.

There are other ecto-parasites like the stubby root- and stunt nematodes that also cause problems in tobacco and for them soil counts are necessary. Most companies do their evaluations on the root knot nematode only, but then they should specify this on their label. Then they cannot say that their substance controls nematodes – and that without any counts. Different nematode species react differently to nematicides, e.g. one specific nematicide gave excellent control of a lesion nematode while another had no effect on it whatsoever.

It is so that certain nematicides only disorientate the nematodes, but hungry nematodes quickly perish and then you won't count them anyway.

The farmer will be satisfied with a higher yield, yes, but does your label specify a higher yield or effectivity as a nematicide, and what happens to the follow-up crop if the nematodes are not controlled?

5.3 Visual phytotoxicity ratings at regular intervals, three times during the season. Root damage (or health) should be assessed at the end of the season. Consult with the advisors.

5.4 The biomass of plants (aerial parts and roots) (fresh weight), if the trial is terminated at twelve weeks, alternatively yield in kg/ha must be determined at the end of the growing season.

5.5 Quality of tobacco assessed by the probable financial return (R/ha) after harvesting and curing of the leaves.

5.6 Root gall index at the termination of the trial.

5.7 Rainfall and irrigation figures for the duration of the trial must be supplied.

5.8 Soil moisture content at the time of application of nematicides should be determined.

5.9 Chemical- and texture analysis of soil in the trial area must be supplied. The organic matter (organic carbon) content of the soil should be determined.

5.10 Soil temperature should be recorded in cases where nematicidal action is dependent on temperature.

5.11 A mineralogical analysis of soil may be useful to assist with the interpretation of results.

COMMENTS:

SECTION 3 FIELD TRIALS POINT 5.7 - 5.1 1:

AVCASA:

Some methods employed to generate this information can be very expensive, for example organic carbon, etc and can be of no consequence.

Proposed change:

"it is recommended that additional information for example, rainfall, irrigation, ... and other relevant information be included in the efficacy report."

INDUSTRY:

Agree

SEEDBED TRIALS

4. Minimum plot size should be 2 m^2, of which 1 m^2 may be used for data acquisition.

5. Data acquisition should include the following:

5.1 Nematode counts from representative soil samples from each plot taken:

- 5.1.1 Prior to application of chemicals and prior to sowing of seed;
- 5.1.2 Six weeks after emergence;
- 5.1.3 Twelve weeks after emergence.

5.2 Nematode counts from representative root samples from each plot taken:

- 5.2.1 Six weeks after emergence;
- 5.2.2 Twelve weeks after emergence.

COMMENTS

SECTION 3 SEED BED TRIALS POINT 5.1 AND 5.2

AVCASA

"Nematode count from representative soil samples must be done by a qualified nematologist directly before the trial begins. This is required to confirm the presence and numbers of plant parasitic nematodes."

In most cases the nematode count data does not justify the high costs involved. There is a tendency evolving within research institutions (outside and within the ARC) that nematode counts are considered less and less important in nematicide efficacy trials. Some institutions even regard it as optional.

The questions that should be asked are, what is our goal, when doing nematode counts and are we achieving it?

INDUSTRY:

Statistically proven results must show that the PPA is effective against the specific pest.

GENERAL COMMENT

AVCASA:

Let us take the pre-trial soil count and later soil counts as examples:

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Under controlled conditions where artificial inoculations are done, or where a susceptible crop, like beans, are planted to activate and build up nematode populations before a nematode trial commences, nematode counts may be worthwhile. Field trials (for registration purposes) are however not conducted

under ideal conditions and therefore the current emphasis placed on nematode counts should be re-considered, especially taking into account the high costs involved in counts. Several nematicides, at the dosages applied, does not necessary directly kill the nematodes. Control may also be achieved by disorientation of nematodes, damaging the cuticle, enhancing natural enemies, enhancing plant growth and resistance, etc.

At the end of the day it is increased yield and quality of the crop that are the important parameters. Farmers are not satisfied with reduced nematode counts after applying a nematicide, they are only satisfied with increased crop yield and/or quality. The use of other parameters to establish the presence of nematodes, like root gall indexes with root knot nematodes for example, can also substitute counts.

It is long overdue for us to take into count the high cost involved in doing nematode counts and ask ourselves: Would not our end purpose be served equally well by substituting nematodes counts with other suitable parameters? Or by only establishing the presence and numbers of nematodes only with one nematode root count (after planting) in control plots? Or doing only one nematode root count in all plots, for example six or twelve weeks after planting? Please comment.

INDUSTRY:

Nematodes have a devastating effect on tobacco and one must be certain that you are controlling the pest. Nematode numbers should be determined at the end of the previous crop when they do occur in the root zone.

There are other ecto-parasites like the stubby root- and stunt nematodes that also cause problems in tobacco and for them soil counts are necessary. Most companies do their evaluations on the root knot nematode only, but then they

should specify this on their label. Then they cannot say that their substance controls nematodes – and that without any counts. Different nematode species react differently to nematicides, e.g. one specific nematicide gave excellent control of a lesion nematode while another had no effect on it whatsoever.

It is so that certain nematicides only disorientate the nematodes, but hungry nematodes quickly perish and then you won't count them anyway.

The farmer will be satisfied with a higher yield, yes, but does your label specify a higher yield or effectivity as a nematicide, and what happens to the follow-up crop if the nematodes are not controlled?

5.3 Visual phytotoxicity rating at one, two and four weeks after seedling emergence. Emergence tests should be done prior to sowing of seed.

5.4 Plant population per square metre at termination of trial.

5.5 Biomass of plants (aerial parts and roots) at the termination of the trial.

5.6 Number of plants suitable for transplanting per square metre.

5.7 Root gall index at the termination of the trial.

5.8 Rainfall and irrigation figures for the duration of the trial must be supplied.

5.9 Soil moisture content at the time of application of nematicides must be determined.

5.10 Results of chemical and texture analyses of soil in the trial area must be supplied. Organic matter (organic carbon) content of the soil should be determined.

5.11 Soil temperature must be recorded in cases where nematicidal action is influenced by soil temperature.

COMMENTS

SECTION 3 SEED BED TRIALS points 5.7 - 5.1 1:

AVCASA:

Some methods employed to generate this information can be very expensive, for example organic carbon, etc and can be of no consequence.

Proposed change:

"it is recommended that additional information for example, rainfall, irrigation, ... and other relevant information be included in the efficacy report."

INDUSTRY:

Agree

5.12 Mineralogical analysis of soil may be done to assist with the interpretation of results.

EVALUATION OF BIOLOGICAL CONTROL AGENTS OF NEMATODES OR OTHER NEMATODE SUPPRESSANTS WITH A BIOLOGICAL IMPACT

The same protocol as described above should be used for all biological control agents, but consultation with advisors is of utmost importance. The species names of all organisms must be given. Full details of other nematode suppressants must be given. Additional data required:

1. The total nematode population, must be determined in all soil and root samples. Record the number of plant-parasitic and saprophytic (fungivores/bacteriovores/predators) nematodes separately.

2. All information regarding the soil environment, including organic matter, must be provided.

3. When a biological control organism is introduced to the soil, soil samples must be analysed six weeks after the introduction, to indicate the presence of the specific organism.

SECTION 4: HERBICIDES

1. Results of two types of experiments must be submitted for each product, dosage level and formulation. This must be from trials for:

1.1 Herbicidal efficacy.

1.2 Phytotoxicity to the tobacco crop and following crops.

These trials should be done separately.

COMMENTS

SECTION 4: POINT 1:

AVCASA:

Why should the efficacy and phytotoxicity trials be conducted separately? This is not a common practice.

INDUSTRY:

Efficacy and phytotoxicity trials cannot be conducted together. In phytotoxicity trials the plots have to be kept weedfree so that the weeds do not affect the growth of the crop. In efficacy trials one determines which weeds are influenced at what stage by the herbicide treatment.

2. The success of each agent as well as phytotoxicity symptoms may be illustrated with photographs showing treated and untreated plots.

3. Where applicable, the comparative efficacy of different methods of application, i.e. high volume, low volume, ultra low volume, aerial, etc. should be demonstrated.

COMMENTS

SECTION 4 POINT 3:

AVCASA

Is ultra low volume not more appropriate in the sections dealing with insecticides?

INDUSTRY:

ULV is not a general practice with herbicides but can not be ignored completely.

4. Apart from determining the efficacy of the herbicide for weed control or the effect of the growth regulant on the plant, the effect of the treatments on the tobacco yield and quality of the cured leaf should also be determined.

   Data on the following parameters must be included: Plant height, number of leaves per plant, days to flowering, chemical composition of leaves and leaf quality indicated by the average price per kg given by a leaf grader from one of the tobacco co-operatives.

   Depending on the tobacco variety, 16-20 leaves should usually be harvested per plant. Leaf length and width (in mm) of the 4 th, 8 th, 12 th, and 16 th leaf from five data plants per plot should be taken at the time of harvest. **The effect of the PPA on the taste** of the tobacco must be determined by smoke tests (taint trials).

COMMENTS

SECTION 4: POINT 4:

AVCASA:

"Data on the following parameters must be included.....”

AVCASA

Proposed change:

"Data on the following parameters must be included ..... where applicable “

INDUSTRY:

We agree.

COMMENTS

SECTION 4 POINT 4.

AVCASA:

"The effect of the PPA on the taste.....”

(See comments on SECTION 7: TAINT TESTS)

What about pre-plant contact herbicides for example?

INDUSTRY:

We should discuss this at the follow-up meeting between the Industry and AVCASA.

EFFICACY TRIALS

1. Test sites with a variety of common weeds, should be selected. In the case of post-emergence treatments, weed size, **their biomass** or percentage of ground cover by each weed species must be noted before application of the herbicide treatment. After application, the site should be visited at regular intervals and the percentage weed kill or growth retardation and the spectrum of weeds that were controlled, noted.

COMMENTS

SECTION 4: EFFICACY TRIALS, point 1:

AVCASA:

" .... their biomass... "

Determining biomass in trials is impractical.

INDUSTRY:

We agree – it should be removed.

2. Adjacent to each plot there must be a small untreated control area which can be used for weed control evaluation.

3. In trials with growth regulants, the plots must be kept weed free. If necessary, regular hand-weeding, careful mechanical cultivation, or a selective registered herbicide may be used. An even stand of tobacco must be selected. Plots should have at least 10-15 plants to ensure that 5-10 plants of equal size are available for data collection. **There should be a minimum of two guard**

plants on either side of the data row. If a double row is used, each row should have guard plants. Treatments should be replicated at least four times.

COMMENTS

SECTION 4: EFFICACY TRIALS, point 3:

AVCASA:
"There should be a minimum of two..... four times"

Please clarify the meaning of the last sentences of paragraph.
(See also comments on SECTION 1, 2 and 3 TRIAL LAY-OUT.)

INDUSTRY:
Perhaps "A minimum of two non-data plants should be planted on both ends of each data row to equalise growing conditions of the data plants within the row."

"The minimum number of replications needed for statistical analysis should be determined by the number of treatments".

4. The candidate PPA should initially be applied at varying dosage rates which will permit the establishment of a threshold efficacy level and the optimum dosage rate. Lower and higher rates than the expected dosage rate must be applied.

COMMENTS

SECTION 4: EFFICACY TRIALS, point 4:

AVCASA:

"Lower and higher rates than the expected dosage rate should be applied."

Why should a higher rate be applied if the phytotoxicity trials must be done separately ? See also comment on Section 4, point 1. What about a generic registration?

INDUSTRY:
Perhaps we can again say: "One to several dosages could be included in the first trial and then reduced during further development". This would probably also apply to generic compounds.

5. The most generally used PPA with a similar spectrum of control or effect should be included as a standard for comparison.

6. Efficacy may be supported by photographic evidence.

7. Control of suckering should be assessed by counting the total number of suckers per plant on 10 data plants per plot at intervals of 1-, 2-, 4-, and 6 wk after application of the PPA. All suckers should be removed from the plants 6 wk after application. The dry weight of suckers per plant per replicate should be determined by drying and weighing all the suckers per replicate.

COMMENTS

SECTION 4. EFFICACY TRIALS, point 7:

AVCASA:
Why should the dry weight of suckers be determined and how should it be dried?

What is wrong with wet weight?

INDUSTRY:

It is more accurate to work with dry mass because the moisture status of fresh material can vary. Dry at 60ºC until a constant mass is reached.

PHYTOTOXICITY

1. It is imperative that trial plots are kept free of weeds during the entire trial period.

2. Phytotoxicity studies should be done on different cultivars of the main cultivar types grown in South Africa. **The phytotoxicity of registered herbicides to new cultivars will be determined by the breeder before they are released for commercial production.**

COMMENT

SECTION 4 PHYTOTOXITY, POINT 2.

AVCASA:

"The phytotoxity of registered herbicides to new cultivars will be determined by the breeder before they are released for commercial production."

Who is going to pay for this? Will the breeder take legal responsibility when herbicide damage occurs in the field on his new cultivar? Will all breeders

within the ARC and outside (like at the tobacco co-operations) accept responsibility for this?

INDUSTRY:

We think this point should be left out – it could cause problems.

3. The method of application should be the same as for the efficacy trials. Treatments must include at least the expected application rate for registration and double that rate, as well as an untreated (hand-weeded) control.

COMMENTS

SECTION 4 PHYTOTOXITY, POINT 3:

AVCASA:
See comment on Section 4 - HERBICIDES, point 1.

INDUSTRY:
Answer given

COMMENT ON SECTION 4: POINT 1:

AVCASA:
Why should the efficacy and phytotoxicity trials be conducted separately?
This is not a common practice.

INDUSTRY:

Efficacy and phytotoxicity trials cannot be conducted together. In phytotoxicity trials the plots have to be kept weedfree so that the weeds do not affect the growth of the crop. In efficacy trials one determines which weeds are influenced at what stage by the herbicide treatment.

4. Visual signs of phytotoxicity, if any, must be assessed at regular intervals throughout the active growing period. The effect on yield, changes in growth rate, plant height and/or dry biomass produced during the active growing period, should be recorded.
5. A description of the phytotoxicity symptoms supported by photographic evidence should be given. The recovery of plants after initial phytotoxic effects must be recorded and described as well.

COMMENT

SECTION 4 PHYTOTXITY POINT 5:

AVCASA:

Photographic evidence should be a recommendation not a requirement.

INDUSTRY:

What about: "A description of the phytotoxicity symptoms, <u>which could be</u> supported by photographic evidence should be given."

6. Where applicable, e.g. in the case of dinitroanilines, the residual effect of herbicides should be determined and the minimum safe period for tobacco or other following crops in a rotation system, be specified. **The residual effects must be determined on three different soil types by applying the recommended and double the recommended rate and an untreated control, in strips on a suitable field. Other crops usually grown in rotation with tobacco should be planted in these trials.**

COMMENTS

SECTION 4 PHYTOTOXITY POINT 6:

AVCASA:

"The residual effects must be determined..... Should be planted in these trials."

"Where applicable" should be added to this sentences.

INDUSTRY:

May be changed as suggested.

SECTION 5: PHYTOTOXICITY

1. The purpose of these trials are to determine to what extent **any** experimental agent can be damaging to the plant when applied to different cultivars growing in various climatic conditions (different areas), at single- and double dosage rates.

2. All PPA's for fungus-and, bacterial diseases, nematodes-, insects- and mites, as well as growth regulators and herbicides that are applied to tobacco in any way, must be tested for phytotoxicity. Phytotoxicity trials with herbicides are described in Section 4.

TRIAL LAY-OUT

1. The trials for testing phytotoxic effects should comply with the following basic requirements:

1.1 An untreated control.

1.2 The application of the expected dosage for registration at single and double rates

2. Four replications per treatment.

3. The most important commercial cultivars should be used in these trials.

EVALUATION

1. Record any abnormality in size, shape, and colour of any part of the plant.
2. Assess the effect of the products on plant growth by means of a plantgrowth vigour index. Record the data 2 wk after every application of the agent.

COMMENTS

SECTION 5. EVALUATION, POINT 2:

AVCASA:

"Assess the effect...." and "Record data 2 wk after every application of the agent."

Please define and include an example of a plant vigour index.
This period will vary according to the product, its mode of action, etc.

INDUSTRY:

With “vigour index” is meant the visual condition of the treated plant in relation to that of the untreated control plants. An example of a vigour index could be a 10 point scale where the control plant has a value of 5.

SECTION 6: RESIDUE ANALYSES

(Consult the latest requirements of Registrar Act 36/1947)

1. The purpose of these analyses are to determine the residue level on/in the plant at the time of harvesting. The Coresta Chemical Residue Committee standards for maximum permissible residues in tobacco applies in this instance.

2. Phytotoxicity- and efficacy trials as well as the taking of samples for residue analyses can be done on the same experimental plants at the same sites.

3. Dosages and applications :

3.1 Recommended dosage at the maximum number of applications at approximately 12 wk after transplanting.

3.2 Double the recommended dosage at maximum number of applications at approximately 12 wk after transplanting.

3.3 An untreated control.

4. Plots should each preferably consist of at least 4 data rows with 2 border rows. With rows of 20 m in length, sufficient material should be available.

5. Samples should preferably be taken in a commercial field where the compound was applied with standard apparatus.

COMMENTS

SECTION 6: POINT 5.

AVCASA:

Before a product is registered, samples can only be taken from field trials, since the product may not be applied commercially at that time.

INDUSTRY:

Leave out the word "commercial".

6. Green samples (to be frozen) as well as samples of leaves, which have been through the flue-curing-/air-curing-/sun-curing process, must be taken.

7. Sampling:

7.1 Where chemicals are sprayed on plants:

7.1.1 Green samples for freezing:

Take representative green leaf samples (∀ 1 kg) from plots with all dosages 3 h after the last spraying, at about 10-12 wk after transplanting and then at intervals of 1 d, 2 d, 4 d, 8 d, 16 d, 24 d and 32 d after spraying for collecting data to the break down curve. These samples should be stored in a freezer immediately after collection until they are analysed.

Take representative green leaf samples (∀ 1 kg) from plots of all dosages at about 14-, 16- and 18 wk after transplanting, depending on the maturing of tobacco leaves, and store them in a freezer immediately after collection until they can be analysed.

COMMENT

SECTION 6., POINT 7.1.1. & POINT 7.3.1:

AVCASA:

The two paragraphs are contradictory to each other. Please clarify. This contradicts the Agricultural Remedies Residue Data Requirements Document as different requirements exists for new active ingredients, different formulations, different sources of a.i.'s ect. The. requirements are also more strict than that of an edible product. When should green samples be taken 12 wk after transplanting or rather at commercial harvest?

Proposed change.
Maximum 4 - 5 sampling dates are required for the breakdown curve in the case of a systemic product and one green and cured sample in case of a non systemic product. Clearly the cost factor has not been taken into account during the writing of this protocol, for example in the case of a generic product?

7.1.2 Flue-cured samples:

Harvest maturing tobacco leaves (∀ 10 kg) for flue-curing from plots of all dosages at about 14-, 16- and 18 wk after transplanting.

7.1.3 Air-cured samples:

Harvest **enough plants** from plots of all dosages when ready for air-curing.

COMMENT

SECTION 6. POINT 7.1.2. , 7.1.3 & POINT 7.3.2, 7.3.3

AVCASA:
Is all this necessary and logical? What if residue levels are not detectable before harvest on green samples, or at first sampling date, or in the cured product?

Surely only the sample where residues will most likely be detectable (probably the cured sample when residues are concentrated) should be analysed at first. Other samples should only be analysed if detectable residues are found. Once again, the cost factor has been ignored?

Will not the cultivar determine if the end product will be flue or air-cured? It is usually either the one or the other method, not both. What purpose would it serve to flue-cure a cultivar and test if for residues, if the cultivar is only cultivated for air-curing.

Should it not rather be specified that, for example two flue-cured cultivars and one air-cured cultivar should be used in residue trials?

7.2 Chemicals applied as a dust:

The same guidelines as for chemicals sprayed on plants should be used, but plots should be doubled in size with **more border rows** to prevent cross contamination.

COMMENTS

SECTION 6. POINT 7.1.3. and 7.2..
AVCASA:

How many are "enough plants...." and "more border rows .... "

7.3 Chemicals applied to the soil:

7.3.1 Green samples for freezing:

Apply the compound at the **appropriate time - usually at transplanting.** Take representative green leaf samples (∀1 kg) from plots of all dosages when

enough material is available (from small plants) and weekly thereafter up to about 18 wk after transplanting, depending on maturing of the tobacco leaves. These samples should be stored in a freezer immediately after collection until they are analysed.

Take representative green leaf samples (∀ 1 kg) from plots of all dosages at about 14-, 16- and 18 wk after transplanting and store in a freezer immediately after picking until analyses can be done.

COMMENTS

SECTION 6 POINT 7.3.1:
..... appropriate time - usually at transplanting

AVCASA:

Proposed change:
"usually before, or at transplanting ....

SECTION 6., POINT 7.1.1. & POINT 7.3.1:

AVCASA:
The two paragraphs are contradictory to each other. Please clarify. This contradicts the Agricultural Remedies Residue Data Requirements Document as different requirements exists for new active ingredients, different formulations, different sources of a.i.'s ect. The. requirements are also more strict than that of an edible product. When should green samples be taken 12 wk after transplanting or rather at commercial harvest?
Proposed change.
Maximum 4 - 5 sampling dates are required for the breakdown curve in the case

of a systemic product and one green and cured sample in case of a non systemic product. Clearly the cost factor has not been taken into account during the writing of this protocol, for example in the case of a generic product?

7.3.2 Flue-cured samples:

Harvest maturing tobacco leaves (∀ 10 kg) for flue-curing from plots of all dosages at about 14-, 16- and 18 wk after transplanting..

7.3.3 Air-cured samples:

COMMENT

SECTION 6. POINT 7.1.2. , 7.1.3 & POINT 7.3.2, 7.3.3

AVCASA:
Is all this necessary and logical? What if residue levels are not detectable before harvest on green samples, or at first sampling date, or in the cured product?

Surely only the sample where residues will most likely be detectable (probably the cured sample when residues are concentrated) should be analysed at first. Other samples should only be analysed if detectable residues are found. Once again, the cost factor has been ignored?

Harvest enough plants from plots of all dosages when ready for air-curing.

8. Samples should not come into direct contact with each other.
9. Use only paper bags for sampling.

COMMENTS

SECTION 6, POINT 9:

AVCASA:
Freezing samples in paper bags are impractical.
Please provide more detail on sample protocol.

GENERAL COMMENT ON SECTION 6

INDUSTRY:
Much has been said about the whole Residue section. The Tobacco Industry only wants to ensure that the residues are acceptable to the Registrar. We suggest the following replace the Residue section:

SECTION 6: RESIDUE ANALYSES

1. **Residue data should be collected according to the "Agricultural Remedies Residue Trial Data Requirements Document" of August 1998 (Registrar: Act No. 36 of 1947).**

2. **The Coresta Chemical Residue Committee standards for maximum permissible residues in tobacco should be used as guideline.**

3. **Green samples (to be frozen) as well as samples of leaves, which have been through the flue-curing, air-curing or sun-curing process, depending on the type of tobacco on which the PPA is to be registered, should be taken for residue analysis.**

SECTION 7: TAINT TESTS

1. The purpose of these tests are to determine whether any unacceptable taste can be noticed when a cigarette, made from tobacco treated with the experimental compound, is smoked. **Taint tests are also required for generic compounds.**

2. All PPA's for fungus- and bacterial diseases, nematodes, insects and mites. All fungus-, bacterium-, nematode-, insect- and mite agents as well as herbicides and growth regulants applied to tobacco in the field and which may in any way result in an unacceptable taste of the processed tobacco, must undergo taint tests.

COMMENTS

SECTION 7: POINT 1 AND 2:

AVCASA:

It is stated in the document that all most everything must undergo taint tests. This is a very expensive exercise required by people who are not responsible for the costs involved. What is the reasoning behind this argument? Were problems encountered with specific products? Several generic compounds have been used for years in tobacco with no resulting problems? Why should a product, for instance undergo taint tests, if no detectable residues was found? Or a generic a.1. already used for years? What about the role of the all most 60 registered adjuvants and the role of combinations of products ect? Where will this end and what is a practical?

Too severe (and sometimes unnecessary) requirements will result in companies not registering generic and new products in tobacco. At the end it will be the tobacco industry that will suffer the most due to the lack of registered agrochemicals.

Should not taint tests be limited to products leaving detectable residues in the final product and with certain identified problematic products? Why should double rates be included? Isn't this a case of "over killing" ?

INDUSTRY:

It may be true that it is expensive, but not long ago two contact nematicides (according to the manufacturers) did influence the taste of the cigarettes. These were products that did not leave residues. We think this point should be discussed.

TRIAL LAY-OUT

1. The following treatments should be included:

1.1 An untreated control.

1.2 The expected dosage for registration at single and double rates.

2. Plots should be large enough to provide at least 5 kg of cured tobacco for every treatment (approximately 15 data rows and two border rows of 50 m, depending on barn-size)

3. Harvest mature leaves and flue-cure/air-cure treatments separately.

4. Grade the cured tobacco to various grades and keep the treatments separate.

5. For trial cigarettes, use **only** the grades that are present in **all** the treatments

and mix them in the same ratio for **all** the treatments.

6. Cut the tobacco and make cigarettes.