

SUBMITTED VERSION

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Nitrogen supply and sink demand modulate the patterns of leaf senescence in maize

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Manuscript Details

Manuscript number	FIELD_2018_160_R1
Title	Leaf senescence patterns in maize are modified by nitrogen supply and sink demand in a sub-humid tropical environment
Article type	Research Paper

Abstract

Senescence is a key physiological process that can regulate crop grain yield. Patterns of leaf senescence and its association with grain yield for a short maturity maize hybrid were investigated in a factorial combination of two tillage treatments (conventional and no-till), three amounts of stubble (0, 3 and 5 t ha⁻¹) and three N rates (0, 80 and 120 kg N ha⁻¹) over three seasons in 2015 long rains, 2015/2016 short rains and 2016 long rains. Leaf senescence from flowering to harvest was assessed at (a) the whole-plant scale by the visual scoring of dry leaves and (b) the canopy-layer scale by measuring leaf greenness with a SPAD 502 chlorophyll meter. A bilinear model was used to quantify the patterns of senescence at the whole-plant scale. A logistic function was fitted to estimate the traits of senescence at three canopy layers (top, mid, bottom), including minimum and maximum SPAD, onset of senescence (EC90), time to loss of 50% maximum SPAD (EC50) and the rate of senescence in each layer. Nitrogen rate effect on patterns and traits of senescence were large and its interactions with stubble were more frequent than interactions between other treatments. Tillage and stubble amount had marginal effects. EC50 was delayed in the unfertilized controls compared with fertilized crops and was negatively correlated with grain yield. Rate of senescence was faster in fertilised crops compared with unfertilized controls at both whole-plant and canopy-layer levels. Grain yield, grain number and nitrogen remobilization efficiency were associated with faster rates of senescence in the top and mid leaves but with slower rates of senescence in the bottom layer leaves. We advance a sink-driven leaf senescence ideotype for high yield and efficient use of nitrogen for short maturity maize.

Keywords	no-till, stubble, source-sink, N remobilization efficiency, SPAD
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Suggested reviewers	Jos Schippers, James Hunt, John Kirkegaard, Jack Christopher

Submission Files Included in this PDF

File Name [File Type]

Letter to Editor Field Crops Research 20180518.docx [Cover Letter]

Response to reviewer comments.docx [Response to Reviewers]

Highlights.docx [Highlights]

Kitonyo et al. - Revised manuscript WITHOUT TRACK CHANGES.docx [Manuscript File]

Kitonyo et al. - Figures and Tables.docx [Figure]

Kitonyo et al. - Revised manuscript with track changes.docx [Supporting File]

To view all the submission files, including those not included in the PDF, click on the manuscript title on your EVISE Homepage, then click 'Download zip file'.

Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
Data will be made available on request

Prof Chris van Kessel
Chief Editor
Field Crops Research

18 May 2018

Dear Prof van Kessel,

This letter accompanies the revised manuscript entitled **“Leaf senescence patterns in maize are modified by nitrogen supply and sink demand in a sub-humid tropical environment”** which we would like you to consider further for publication in *Field Crops Research*.

This manuscript is a separate work that complements our recently accepted manuscript in *Field Crops Research*, entitled:

“Nitrogen fertilization modifies maize yield response to tillage and stubble in a sub-humid tropical environment”.

The current article focuses on the importance of senescence and its impact on maize yields in smallholder farming systems in relation to tillage, stubble and nitrogen supply and timing in affecting. We believe the paper would be of great interest to the readership of *Field Crops Research* given the importance of senescence as a physiological process impacting grain yield. Our paper provides some robust experimental evidence to understand the interactions across different seasonal conditions in Kenya.

The authors declare that the manuscript is an original work and has not been submitted for publication elsewhere. We confirm that there are no known conflicts of interest associated with this publication.

Substantial changes have been made to the manuscript, based on the comments from the reviewers. We note that many of the comments from the reviewers were based on the fact that the initial publication was not accepted. The first article has now being published in *Field Crops Research*.

Thank you for your consideration. We look forward to further correspondence with you.

Sincerely,



Dr Matthew Denton
Senior Lecturer

RESPONSE TO REVIEWER 1

The paper assessed leaf senescence in maize using a visual score and SPAD chlorophyll meter, across no-till vs, conventional and three stubble amounts and three N rates. The aim was to characterize the time-course of leaf senescence during grain filling and establish relationships between senescence and grain yield, yield components, crop N status as quantified by NNI, and traits related to nitrogen use efficiency. The research found that Tillage and stubble amount had marginal effects. EC50 was delayed in the unfertilized controls compared with fertilized crops and was negatively correlated with grain yield. Rate of senescence was faster in fertilised crops compared with unfertilized controls at both the whole-plant and canopy-layer scales.

Question 1:

Generally the paper was well written. The main issue is that the methods are not described adequately, especially as they refer to an associated paper that has not yet been published and was not available to me (it is under review). Therefore I found it rather difficult to follow parts of the results and discussion. For example Figure 7 shows High and Low yield graphs, without a suitable explanation of where this high and low yield came from – was it the high N and low N etc.

Reply: At this point, the second submission can now cite Kitonyo et al. 2018, which has been published in Field Crops Research. ***“Kitonyo, O.M., Sadras, V.O., Zhou, Y., Denton, M.D., 2018. Nitrogen fertilization modifies maize yield response to tillage and stubble in a sub-humid tropical environment. Field Crops Research 223, 113-124”*** It is now unnecessary to duplicate the materials and methods section, hence we refer readers to the preceding

publication. In the present submission, materials and methods section has been expanded for detailed description.

Question 2:

I note also that the low N has a slower rate of senescence than the high N in the top and middle leaves, yet (as expected) the level of greenness (proxy for chlorophyll) is nearly always lower. Is this relevant?

Reply: This study highlights leaf greenness particularly when describing the senescence ideotype. However, rate of senescence is an important feature. The results are relevant in that low N crops had slower rates of senescence compared with fertilized counterparts. This largely contradicts previous literature that shows that the rate of leaf senescence is accelerated under low N conditions. The study discusses the potential mechanisms that were responsible for the slower rate of senescence in low N crops.

Question 3:

The authors conclude “that patterns of senescence are valuable traits to explore in the improvement of grain yield and quality in conservation agriculture.” – yet there is little evidence here to back up that statement for conservation agriculture – there was little difference between conservation agriculture and conventional agriculture.

Reply: The second submission has reduced emphasis on conservation agriculture by being specific on the treatments tested: tillage, stubble and N rate. Results showed that the effects of tillage and stubble retention marginally affected the patterns of senescence in maize but

that senescence was significantly impacted by N rate. The conclusions have therefore been revised.

Question 4:

Need more information in the methods, especially as the other paper is yet to be published – insufficient information for the reader to evaluate the NT vs conventional. What did the residue consist of, what form was the fertilizer. Were all the 3 seasons/trials on the same piece of land (one after the other) or in different fields?

Reply: In the second submission, materials and methods section has been revised to provide more details on tillage, stubble and N treatments.

In the first submission ‘section 2.2 Treatments and experiment design’ stated:

“Effects of conventional tillage (CT) and no-till (NT), three amounts of stubble (0, 3 and 5 t ha⁻¹) and three fertilizer N rates (0, 80 and 120 kg N ha⁻¹) were evaluated under continuous maize cropping over three seasons. Experiments were laid out as split-split plot design with three replications. Tillage systems formed the main plots, stubble amount the sub-plots and N rate formed the sub-sub plots. The main plots measured 31 m × 12.25 m, 28 m x 3.75 m for sub-plots while the sub-sub plots were 7 m × 3.75 m.

A locally adapted short-maturity DeKalb (DK) 8031 maize hybrid was used. DK8031 has a fast dry-down syndrome and has a yield potential of 5-8 t ha⁻¹ (Monsanto, 2017). Crops were sown at the onset of rains: on 25th March for 2015 long rains season, 19th October for 2015/2016 short rains season and 3rd April for 2016 long rains season. Crops were sown with

0.75 m between rows and 0.25 between plants within a row to give an approximate density of 5.3 plants m⁻².”

In the revised submission ‘section 2.2 Treatments and experiment design’, in L135-L161, states:

“Effects of conventional tillage (CT) and no-till (NT), three amounts of maize stubble (0, 3 and 5 t ha⁻¹) and three fertilizer N rates (0, 80 and 120 kg N ha⁻¹) were evaluated under continuous maize cropping over three seasons during 2015 long rains, 2015/2016 short rains and 2016 long rains. Tillage and stubble treatments were applied two weeks before sowing in the same plot in all seasons. Prescribed amounts of stubble were supplied in the first season while in the following two seasons, additional stubble allowed for undecomposed material. In CT plots stubble was chopped to small pieces and incorporated into the soil by digging to 15 cm depth while the NT treatments were not disturbed and stubble was surface applied without cutting. Fertilizer N was sourced from urea (46%) and side-banded on the sowing rows in the fractions of $\frac{1}{3}$ at sowing and $\frac{2}{3}$ at six-leaf stage. All plots received 60 P kg ha⁻¹ of basal fertilizer of triple super phosphate which was side banded on the sowing rows.

Experiments were laid out as split-split plot design with three replications. Tillage systems formed the main plots, stubble amount the sub-plots and N rate formed the sub-sub plots. The main plots measured 31 m long and 12.25 m wide, 28 m x 3.75 m for sub-plots while the sub-sub plots were 7 m x 3.75 m. In all plots, sowing was done manually by opening 5 cm deep holes to hold seed and fertilizer.

A locally adapted short-maturity DeKalb (DK) 8031 maize hybrid was used. DK 8031 has a fast dry-down syndrome and has a yield potential of 5-8 t ha⁻¹ (Monsanto, 2017). Crops were sown at the onset of rains: on 25th March for 2015 long rains season, 19th October for 2015/2016 short rains season and 3rd April for 2016 long rains season. Crops were sown with

0.75 m between rows and 0.25 between plants within a row to give an approximate density of 5.3 plants m⁻². “

Question 5:

Leaf layers are not randomly assigned in the ANOVA and this needs to be considered in the statistical analysis.

Reply: In second submission, L233-234 clarifies that leaf layers were not randomly assigned in the analysis of variance.

Question 6:

Nothing about yield measurement mentioned in the measurements/methods and high vs low yield (e.g. Fig 7) – are these the high vs low/0 N. This needs to be more fully explained. Therefore it was difficult to follow what was done for the yield analysis. The authors reference Kitonyo et al. (under review), but I have no knowledge of this as a reviewer.

Reply: In the revised submission, yield measurement has been explained. L218-223 of the second submission explains the design of the senescence ideotype and clarifies high vs. low yield. Further, the caption of Fig. 7 elaborates the high vs low yield, whereby high yield is the grain yield with the addition of fertilizer N while low yield is grain yield without the addition of N.

Such a study is important to understanding factors affecting yield formation in maize. However, there are two major problems.

Question 1:

Firstly, the paper relies heavily on quoting ‘Kitonyo et al. under review’. This is essentially an unpublished draft, that cannot be accessed to assess the claims made. Either the present paper should be delayed at least until ‘Kitonyo et al. under review’ is formally ‘accepted’ for publication or the relevant information from that paper needs to be included in the present paper.

Reply: Kitonyo et al. (2018) has now been published in Field Crops Research. Reviewers and readers are referred to this preceding publication particularly for further details on experiments, that were not described in the present paper to minimise duplication.

Question 2:

Secondly, there is no consideration of how soil moisture status interacted with senescence, and thus modulated the effect of other studied factors, realizing that moisture stress is a major determinant of senescence. Additionally, possible effects of disease and nutrient effects other than N on senescence are not dealt with – at least to demonstrate that they did not impact this study.

Reply: In the revised submission, soil moisture, which could have impacted water use, was quantified as seasonal evapotranspiration. This study found that evapotranspiration did not affect traits of senescence. Also the effects of diseases, pests and nutrient deficiency, other than N in some cases, did not affect the present study.

In the second submission, L224-227 was added to support measurements for evapotranspiration. It states: “As detailed in Kitonyo et al. (2018), water use was estimated as the seasonal evapotranspiration, the change in soil water content between sowing and harvesting plus rainfall during the same period (French and Schultz, 1984). A correlation matrix was used to analyse the impact of evapotranspiration on the patterns of leaf senescence”.

In the results section, L344-348 states: “In Kitonyo et al. (2018), tillage system, stubble amount and N rate did not affect seasonal evapotranspiration, while in the present study evapotranspiration did not alter the traits of senescence. In addition, crops were protected with fungicides and insecticides, thus disease and pest damage did not affect this study. Moreover, apart from varying rates of N supply, crop deficiency from other nutrients were not observed”.

In the discussion, the following statements were added, L355-357: “In addition, sink size was dependent on N supply but the effects of tillage, stubble and many treatment interactions, and seasonal evapotranspiration were small”.

Introduction

Question 3:

P5. ‘Kitonyo et al. under review’ is still a draft, apparently submitted somewhere. Seems premature to include it as a reference.

Reply: Kitonyo et al. 2018 has now been published in Field Crops Research. To minimise duplication of materials and methods, the present paper refers the reader to the published paper, Kitonyo et al. 2018.

Materials and Methods

Question 4:

P7. L115. Again, as ‘Kitonyo et al. under review’ is still an unpublished draft, you need a full explanation of M&M here.

Reply: In the second submission, Kitonyo et al. 2018 has been published. To minimise duplication of Materials and Methods, the present paper refers the reader to this publication.

Question 5:

P8. L124-5. The short and long rains both have a duration of five months – needs elaboration.

Reply: In revised submission, it is elaborated that the rainy seasons are five month duration.

In the first submission, L123-125 read: In this environment, there are two rainy seasons; the short rains season from October to February and the long rains between April and August.

In the revised submission, L129-131 states: In this environment, there are two rainy five-month seasons; the long rains that occur between April and August, and the short rains season from October to February.

Question 6

P8 L137. Monsanto (2017) not a proper reference, just an advert page.

Reply: In the second submission, L153, two alternate and more relevant citations have been used, Abate et al. (2017) and Beyene et al. (2017).

Question 7

P8. L139-41. Need more details of seed sowing and tillage method, fertilizer types, rates and application methods, and weed/pest/disease management.

Reply: In the second submission, the materials and methods section has provided more details of tillage method, fertilization, sowing, weed, and pest and disease management. Reference is also made to Kitonyo et al., 2018 for additional information.

Question 8

P9. L155. Again, can't rely on unpublished draft.

Reply: Kitonyo et al. 2018 has been published in Field Crops Research, thus were we refer reviewers to this manuscript.

Results

Question 8

P12. L207-14. Fig. 1. Y axis units? Really need total crop cycle weather data. What was extent of moisture stress? Use ‘mean’ rather than ‘average’ temperature.

Reply: In the second submission, we refer readers to Kitonyo et al. 2018 for the total crop cycle weather data. In the present paper we show rainfall, temperature and evapotranspiration from flowering to harvesting, the phenological phase during which patterns of senescence were studied.

In the revised submission, additional analysis on the impact of water use on the patterns of senescence is described. Methods are described in L224-227 and results in L344-346.

Question 9

L217-18. Again, really can’t rely on an unpublished draft. The present paper needs to be self-contained if the referred to draft remains unpublished.

Reply: Kitonyo et al. (2018) has been published in Field Crops Research.

Question 10

L218. What was the cause of yields being below their potential (5-8 t/ha) – season, rainfall, low N, etc.?

Reply: In the revised submission, L255 highlights that grain yield varied with seasons, thus seasonal rainfall attributed to the low yield potential. In addition to rainfall, the lack of N supply led to low yields.

Question 11

P13. L233-7. Unclear, do you mean in the overall analysis N effects on breakpoint were ns but when seasons were analysed separately they were?

Reply: In the second submission, L260-267 has clarified treatment effects, both across seasons and when seasons were analysed separately.

In the first submission the L231-237 stated: ‘Season ($P < 0.001$) and N rate ($P = 0.003$) affected the rate of senescence, and the effects were larger for slope 2 than slope 1. Tillage ($P > 0.05$) did not affect rate of senescence while stubble amount had effects on slope 1 only ($P = 0.01$). Season ($P < 0.001$), tillage ($P < 0.001$) and stubble ($P = 0.005$) altered the breakpoint between the two slopes but N rate ($P = 0.98$) effects were not observed. However, ANOVA for individual seasons showed that the breakpoint was mostly altered by N rate while tillage, stubble and interaction effects were small (Table 1).’

The revised statement in the second submission L268-276 states: ‘Across seasons, season ($P < 0.001$) and N rate ($P = 0.003$) affected the rate of senescence, and the effects were larger

for slope 2 than for slope 1. Tillage ($P > 0.050$) did not affect rate of senescence while stubble amount impacted slope 1 ($P = 0.010$) but not slope 2 ($P = 0.102$). At the same scale of analysis, season ($P < 0.001$), tillage ($P < 0.001$), stubble ($P = 0.005$) altered the breakpoint between the slope 1 and slope 2 but for individual season analyses, the effects of tillage, stubble and many interactions were small. In the overall analysis, N rate effects on the breakpoint were absent ($P = 0.98$). However, when seasons were analysed separately a lack of N delayed the breakpoint compared with N supply (Table 1)".

Question 12

P14 L266. ‘..... most interesting (not shown).’ If really most interesting why not show?

Reply: In the second submission, L306-314 we have revised the claim that results for EC90 and EC50 were the most interesting. Instead we provide in-depth discussion on the treatment effects on these traits.

Question 13

P15. Fig. 7. How are the shown data points derived? Are they just theoretical? If so, would just straight lines suffice to represent an ideotype?

Reply: The data points in Fig. 7 are not theoretical but the actual SPAD units against thermal time from a few days after flowering through to maturity. In the revised submission, L218-223 clearly describes how the senescence ideotype was designed.

Discussion

Question 14

P18. L356-7. Contradicts previous sentences. Needs elaboration.

Reply: In the revised submission in L401-405 the statements have been revised to remove contradictions.

The revised statement reads: “Other reports show accelerated leaf senescence in response to low grain yield in maize, potentially due to hormonal and nutritional signals in the regulation of senescence (Sadras et al., 2000). Enhanced contents of cytokinins, for example, may delay leaf senescence in maize (He et al., 2005) but the hormone’s association with reduced sink size in maize is poorly understood”.

RESPONSE TO REVIEWER 3

This manuscript describes leaf senescence patterns in maize in an experiment conducted in Kenya with tillage, residue and N fertiliser rates as factors. It found that senescence was most affected by N rate and to a lesser extent residues (sometimes interacting with N) but rarely by tillage. The authors propose that senescence pattern is determined by sink demand, and propose a senescence ideotype for a short maturity maize hybrid. The content is in scope for the journal and the manuscript is well written and well prepared. The subject matter appears reasonable but do not appear to be particularly novel. The conclusions requires some attention, please see comment below. I can find no major problems with the manuscript, and think it suitable for publication pending minor revisions. However, as the study builds and relies upon a paper that is currently under review in Field Crops Research (Kitonyo et al. under review), publication of this manuscript must be conditional on acceptance of the earlier manuscript. If the first manuscript is not accepted, this manuscript would require significant additions in order to be publishable in its own right.

Question 1:

As the study builds and relies upon a paper that is currently under review in Field Crops Research (Kitonyo et al. under review), publication of this manuscript must be conditional on acceptance of the earlier manuscript. If the first manuscript is not accepted, this manuscript would require significant additions in order to be publishable in its own right.

Reply: Kitonyo et al. (2018) is now published in Field Crops Research, thus reviewers and readers of the present paper are referred to this manuscript. Thus, duplication of materials and methods is minimized.

Some minor comments follow below.

Question 2:

L24 suggest change description of tillage types to ‘...combining two tillage treatments (conventional and no-till), three amounts of stubble...’

Reply: In the first submission, L24-25 stated: “.....a factorial experiment combining conventional and no-till, three amounts of stubble (0, 3 and 5 t ha⁻¹) and three N rates (0, 80 and 120 kg N ha⁻¹) over three seasons.

In the second submission, the revised sentence, L25-27 states: “.... a factorial combination of two tillage treatments (conventional and no-till), three amounts of stubble (0, 3 and 5 t ha⁻¹) and three N rates (0, 80 and 120 kg N ha⁻¹) over three seasons in 2015 long rains, 2015/2016 short rains and 2016 long rains”.

Question 3:

L123 delete ‘of’ and change ‘range’ to ‘ranging’

Reply: L123 has been revised; in the first submission, the sentence stated: “with mean annual temperatures of range between 18-24 °C”.

In the revised submission, the sentence, L128-129 now states: “with mean annual temperature of 22 °C”

Question 4:

L144-146 How was timing of maturity/harvest determined? Please describe.

Reply: In the revised submission, the timing of maize maturity has been described by inserting a new sentence. L166-168 states: “Maize maturity was determined by dislodging kernels and observing the development of a black layer at the point of kernel attachment to the cob”.

Question 5:

L146 Change ‘our’ to ‘this’ and provide a reference for calculating degree days in this way

Reply: In the second submission, L165-166 has been revised and a reference for calculating growing degree days is provided, as Bonhomme (2000).

Question 6:

L149-152 Where on the leaf were SPAD measurements taken?

Reply: SPAD measurements were taken at the whole-leaf length; this has been clarified in the second submission at L174

Question 7:

L192-194 confused as to if you used GenStat or SAS or both? If you used SA please include publisher etc.

Reply: SAS was not used in data analysis. Only GenStat and SigmaPlot were used and publishers are now referenced appropriately.

Question 8

L217-226 this manuscript relies on an earlier manuscript still under review, and acceptance must be conditional on acceptance of the earlier manuscript.

Reply: Kitonyo et al. (2018) has been published in Field Crops Research, thus reviewers and readers can refer to this manuscript.

Question 9:

L266 it seems odd to say a particular set of a data are ‘most interesting’ but then not show them! And are these data not shown in Fig 6 d, e, f?

Reply: In the revised submission, L306-314 we have revised the claim that results for EC90 and EC50 were the most interesting. Instead we discuss the treatment effects on these traits.

Question 10:

L278-279 this effect does not appear significant according to the error bar in Fig 6 h & i?

Reply: The effects of N rate on the rate of leaf senescence were larger in 2015 long rains and marginal in both 2015/2016 short rains and 2016 long rains. In Fig. 6 h, the error bar shows that there were differences in the mid and bottom layer leaves while in Fig. 6 i, there were differences in top layer only. In the second submission, L315-317 reports that the differences in the rate of leaf senescence due to N rate were large in 2015 long rains but marginal during 2015/2016 short rains and 2016 long rains.

Question 11:

L280-281 there does appear to be differences in rate of senescence across canopy in first season when N was applied Fig 6 g?

Reply: In the revised submission, L315-317 clarifies that effects of N rate on the rate of leaf senescence varied with season, and that differences were large during 2015 long rains (Fig. 6 g) and marginal in 2015/2016 short rains and 2016 long rains seasons (Fig. 6 h and i).

Question 12:

L281-282 only slower in bottom layer if N is applied?

Reply: In the revised submission, L318 clarifies that the rate of leaf senescence was slower in the bottom layer leaves only when N was supplied.

Question 13:

L414-415 how can you conclude this when your experiment either showed no or very little effect (or interaction) of the two main components of conservation agriculture (no-till and residue) on leaf senescence? Patterns of senescence appear to be determined by sink-size i.e. yield, so how can grain yield be improved by changing senescence pattern based on the evidence you have presented?

Reply: In the revised submission, the conclusions reduce emphasis on conservation agriculture. Instead they are specific about tillage, stubble and N rate.

1 **Highlights**

- 2 • Leaf senescence in maize was studied under combinations of tillage, stubble, N rate
- 3 and seasons
- 4 • Nitrogen altered leaf senescence, but season, stubble and tillage effects were small
- 5 • Sink size regulated senescence in the maize canopy
- 6 • A senescence ideotype is proposed

**Nitrogen supply and sink demand modify the patterns of leaf senescence in the canopy
of maize in a sub-humid tropical environment**

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14 **Highlights**

- 15 • Leaf senescence in maize was studied under combinations of tillage, stubble, N rate
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- 17 • Nitrogen altered leaf senescence, but season, stubble and tillage effects were small
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- 19 • A senescence ideotype is proposed

20

21

Abstract

Senescence is a key physiological process that can regulate crop grain yield. Patterns of leaf senescence and its association with grain yield for a short maturity maize hybrid were investigated in a factorial combination of two tillage treatments (conventional and no-till), three amounts of stubble (0, 3 and 5 t ha⁻¹) and three N rates (0, 80 and 120 kg N ha⁻¹) over three seasons in 2015 long rains, 2015/2016 short rains and 2016 long rains. Leaf senescence from flowering to harvest was assessed at (a) the whole-plant scale by the visual scoring of dry leaves and (b) the canopy-layer scale by measuring leaf greenness with a SPAD 502 chlorophyll meter. A bilinear model was used to quantify the patterns of senescence at the whole-plant scale. A logistic function was fitted to estimate the traits of senescence at three canopy layers (top, mid, bottom), including minimum and maximum SPAD, onset of senescence (EC90), time to loss of 50% maximum SPAD (EC50) and the rate of senescence in each layer. Nitrogen rate effect on patterns and traits of senescence were large and its interactions with stubble were more frequent than interactions between other treatments. Tillage and stubble amount had marginal effects. EC50 was delayed in the unfertilized controls compared with fertilized crops and was negatively correlated with grain yield. Rate of senescence was faster in fertilised crops compared with unfertilized controls at both whole-plant and canopy-layer levels. Grain yield, grain number and nitrogen remobilization efficiency were associated with faster rates of senescence in the top and mid leaves but with slower rates of senescence in the bottom layer leaves. We advance a sink-driven leaf senescence ideotype for high yield and efficient use of nitrogen for short maturity maize.

Keywords: no-till, stubble, source-sink, N remobilization efficiency, SPAD

45

46 **Abbreviations:** NT, no-till; CT, conventional tillage; N, nitrogen; NNI, N nutrition index; NRE,
47 N remobilization efficiency; NUE, N use efficiency; °C, degree celsius; °C d, growing degree
48 day; t, tonnes; ha, hectare; P, phosphorus; kg, kilogram; g, grams; L, litre; DK, DeKalb; DM, dry
49 mass; SPAD, chlorophyll unit; SPAD_{min}, minimum SPAD unit before harvesting; SPAD_{max},
50 maximum SPAD unit before the onset of senescence; EC90, onset of senescence; EC50, time to
51 loss of 50% SPAD_{max}.

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1. Introduction

No-till (NT) management and stubble retention protects the soil from water and soil erosion, improves soil water capture and storage, and promotes soil chemical and physical properties, which leads to higher yields (FAO, 2015). Previous studies on the impact of these practices on crops and cropping systems are restricted to crop growth and yield, water and nutrient economy, with little emphasis on physiological processes (Verhulst et al., 2011; Brouder and Gomez-Macpherson, 2014). Kitonyo et al. (2018) concluded that nitrogen (N) modifies crop response to NT and stubble retention in a sub-humid tropical environment, by altering N nutrition index (NNI), which explained most of the variation in crop growth rate in the critical window of yield determination. Crop growth rate in turn accounted for most of the variation in kernel number and yield while grain yield was linearly correlated with N remobilization efficiency (NRE), which is defined as the difference in shoot N at flowering and harvesting (Kitonyo et al., 2018). The manipulation of patterns of leaf senescence that impact photosynthesis, harvest index and N remobilization, and potentially N use efficiency has received little attention in cropping systems (Masclaux-Daubresse et al., 2010; Wu et al., 2012).

In monocarpic plants such as maize, leaf senescence is a developmental process that involves the gradual loss of green leaf area in the older leaves and finally the whole plant (Gregersen et al., 2013). High yield potential in maize has been achieved through the extension of photosynthetic duration and increased harvest index, two traits that are related with leaf senescence (Bänziger et al., 2002; Wu et al., 2012). Genetic and environmental factors trigger and regulate senescence (Gregersen et al., 2013; Thomas and Ougham, 2015). There is considerable genetic variation in the patterns of senescence in maize (Gregersen et al., 2013). Broadly, senescent hybrids senesce earlier irrespective of growing conditions

while the “stay-green” phenotypes show prolonged green leaf area duration (Gregersen et al., 2013).

The genetic control of senescence is complex and involves both programmed cell death and hormonal regulation (Wilkinson and Davies, 2002; van Doorn and Woltering, 2004; Liu et al., 2005; Lim et al., 2007). Hormonal signals such as abscisic acid, ethylene, cytokinins and jasmonic acid regulate senescence in response to stress and source-sink ratios (Harding et al., 1990; Staswick, 1992; Schippers et al., 2007; Davies and Gan, 2012). Environmental factors like water, nutrient stress and temperature modulate senescence (Gregersen, 2011). Both leaf area duration and green leaf area proportionally impact grain yield (Gregersen et al., 2013). The ratio of assimilate supply (i.e., source) to demand (i.e., sink) during grain filling also regulates senescence, and impacts nutrient fluxes from the senescing leaves to the grain (Feller et al., 2007; Wei et al., 2018). In some species, such as tomato, low source:sink ratios favour senescence but in maize the response of senescence to source:sink ratios varies with hybrid (Crafts-Brandner and Ponelleit, 1987; Sadras et al., 2000).

The translocation of N from senescing tissues to the grain indirectly impacts N use efficiency (NUE) (Masclaux-Daubresse et al., 2008; Gregersen, 2011). At the crop level, NUE, the ratio between grain yield and fertilizer supplied (Dobermann, 2007) depends on N uptake from the soil, internal utilization and the subsequent partitioning and remobilization of N to the grain (Masclaux-Daubresse et al., 2010). Nitrogen remobilisation is fundamental for crop N economy since it controls a large part of N fluxes from sources to sinks (Masclaux-Daubresse et al., 2008). N remobilization efficiency (NRE) accounted for 85% variation in grain yield in both maize (Kitonyo et al., 2018) and wheat (Barraclough et al., 2014). Despite the fundamental role played by N remobilization in crop N economy (Yang

and Udvardi, 2017), patterns of leaf senescence are least explored for the improvement of NUE in NT and stubble retention systems.

In the model advanced by Christopher et al. (2014), the key traits of senescence are quantified: the minimum leaf greenness before harvesting, maximum greenness before the onset of senescence, the timing of onset of senescence, and the progression and rate of senescence. Profiles of leaf senescence vary within the spatial arrangement of leaves, which affects light interception and attenuation, thus shaded leaves often senesce earlier than unshaded ones (Maddonni et al., 2001). In addition, carbon assimilation and N transfer from senescing leaves to the grain varies with leaf position (Feller et al., 2007). The middle leaves and top leaves provide N for grain filling in maize, while the bottom leaves export more N to the roots than to the grain (Feller et al., 2007).

This study investigated the patterns of leaf senescence in maize and their association with yield in a context of NT and stubble retention. Our aims were to (i) characterize the time-course of post-flowering leaf senescence under conventional tillage and NT, and with three stubble and three N rates, and (ii) establish relationships between senescence and grain yield, yield components, crop N status quantified as NNI, and traits related to nitrogen use efficiency.

2. Materials and methods

The experiments are fully described in Kitonyo et al. (2018). Here we briefly summarise treatments and experimental design, and focus on measurements and analysis of senescence.

2.1 Site

A field study was conducted at the Kenya Agricultural and Livestock Research Organisation, Embu research station (0.515 °S, 37.273 °E, 1425 masl), for three consecutive seasons during the 2015 long rains, 2015/2016 short rains and 2016 long rains. Embu is in the upper midland zone three (UM3) and has a sub-humid climate with mean annual temperature of 22 °C (Jaetzold et al., 2006). In this environment, there are two five-month rainy seasons; the long rains that occur between April and August, and the short rains season from October to February. Soils are deep (> 2.5 m) well-weathered humic nitisols with low exchangeable bases and relatively high P-sorption, and of medium to low fertility (Jaetzold et al., 2006).

2.2 Treatments and experiment design

Effects of conventional tillage (CT) and no-till (NT), three amounts of maize stubble (0, 3 and 5 t ha⁻¹) and three fertilizer N rates (0, 80 and 120 kg N ha⁻¹) were evaluated under continuous maize cropping over three seasons during 2015 long rains, 2015/2016 short rains and 2016 long rains. Tillage and stubble treatments were applied two weeks before sowing in the same plot in all seasons. Prescribed amounts of stubble were supplied in the first season while in the next two seasons, additional stubble allowed for undecomposed material. In CT plots stubble was chopped to small pieces and incorporated into the soil by digging to 15 cm depth while the NT treatments were not disturbed and stubble was surface applied without cutting. Fertilizer N was sourced from urea (46% N) and side-banded on the sowing rows in the fractions of ⅓ at sowing and ⅔ at six-leaf stage. All plots received 60 P kg ha⁻¹ of basal fertilizer as triple super phosphate that was side banded in the sowing rows.

Experiments were laid out as split-split plot design with three replications. Tillage systems formed the main plots, stubble amount the sub-plots and N rate formed the sub-sub

plots. The main plots measured 31 m long and 12.25 m wide, sub-plots were 28 m x 3.75 m while the sub-sub plots were 7 m × 3.75 m. In all plots, sowing was done manually by opening 5 cm deep holes with a sharp machete to hold seed and fertilizer.

A locally adapted short-maturity DeKalb (DK) 8031 maize hybrid was used. DK 8031 has a fast dry-down syndrome and has a yield potential of 5-8 t ha⁻¹, and is frequently used as a commercial check in breeding studies in Kenya (Abate et al., 2017; Beyene et al., 2017). Crops were sown at the onset of rains: on 25th March for 2015 long rains season, 19th October for 2015/2016 short rains season and 3rd April for 2016 long rains season. Crops were sown with 0.75 m between rows and 0.25 within plants a row to give an approximate density of 5.3 plants m⁻². Weeds were controlled with Roundup® (glyphosate) before sowing and Dual Gold® (960 g L⁻¹ S-metolachlor) after sowing in both tillage systems while in-crop weeds were removed by hoe-weeding in CT plots but with Twiga 2, 4-D® (Dimethylamine salt and 2, 4-diclorophenoxy acetic acid) in NT plots. Insect pests were controlled with Thunder® (100 g L⁻¹ imidacloprid) at 300 mL ha⁻¹ (Kitonyo et al., 2018).

2.3 Measurements

A phenological scale (CIMMYT, 2017) was used to determine flowering as 50% shedding of pollen. Growing degree days (°C d) were computed as the mean day temperature minus 10 °C, which is the base temperature for maize (Bonhomme, 2000). Timing of maize maturity was determined by dislodging kernels and observing the development of a black layer at the point of kernel attachment to the cob.

Five plants per plot were tagged for the assessment of leaf senescence at the whole-plant scale. Senescence was scored visually from a few days after flowering through to maturity by recording leaves that presented more than 50% green leaf area. At the same

phenological range, vertical profiles of leaf greenness were measured from two tagged plants per plot. From top to bottom leaves, leaf greenness was measured using SPAD 502 chlorophyll meter (Konica Minolta, Japan), at several points of the whole-leaf length and the mean SPAD unit was recorded. Leaf counts and SPAD measurements were sampled at least every two weeks. Even though rare for DK8031, in the circumstances where a plant produced two ears, the lower ear was removed.

Kitonyo et al. (2018) described yield collection and computations for traits related to N use efficiency. Briefly grain yield was measured from a harvest area of 14.6 m², and kernel number m⁻² and 1000 kernel weight were determined in all seasons. Grain protein was measured by near infra-red spectroscopy using FOSS Infratec[®] 1241 grain analyser during 2015/2016 short rains and 2016 long rains only. Nitrogen nutrition index at flowering was calculated as the ratio between the actual N concentration of the shoot biomass and the critical N concentration at the same crop mass (Gastal et al., 2015). Critical N concentration (N_c), the minimum N concentration required to maximise biomass, was computed as: $N_c = 3.4 * (DM)^{-0.37}$; where DM is the actual crop biomass in t ha⁻¹ (Justes et al., 1994; Plénet and Lemaire, 1999). N remobilization efficiency (NRE) was calculated as (N content at flowering – N content at maturity)/N content at flowering.

2.4 Analysis of senescence

At the whole-plant level, rate of senescence was quantified by fitting a bilinear model of leaf counts against thermal time from flowering using the piece-wise routine of SigmaPlot version 10.0 (Systat Software, Inc., San Jose California USA, www.systatsoftware.com) (Eq. 1). This model was used to describe monocarp senescence in fruit (Bonada et al., 2013), and

estimated slope 1 (Eq. 1a), slope 2 (Eq. 1b) and a breakpoint thermal time at the transition from the first to the second slope.

$$Slope\ 1 = \frac{y_1(T - t) + y_2(t - t_1)}{T - t_1} \quad \text{For, } T > t_1 \quad (1a)$$

$$Slope\ 2 = \frac{y_2(t_1 - t) + y_3(t - T)}{t_1 - T} \quad \text{For, } T < t_1 \quad (1b)$$

In Eq. 1, y_1 is number of green leaves at the onset of senescence, y_2 is the number of green leaves at harvesting, y_3 is the number of green leaves at breakpoint, T is the breakpoint (°C d), t is onset of senescence (°C d) and t_1 is the end of senescence (°C d). The bilinear model was fitted for each plot.

The profiles of leaf greenness were separated into three canopy layers: the mid layer of which comprised the ear leaf, one leaf below the ear leaf and two leaves above the ear leaf. The top and the bottom layers consisted of the leaves above and below the mid layer, respectively. A logistic regression function was adopted to fit a time-course of leaf senescence to quantify the traits of senescence at each canopy layer using SigmaPlot (Christopher et al., 2014; Kitonyo et al., 2017) (Eq. 2).

$$y = SPAD_{min} + \frac{SPAD_{max} - SPAD_{min}}{1 + \left(\frac{x}{EC50}\right)^{SR}} \quad (2)$$

Eq. 2 estimated four parameters, including the minimum SPAD before harvesting ($SPAD_{min}$), maximum SPAD before the onset of senescence ($SPAD_{max}$), time to loss of 50% of $SPAD_{max}$ (EC50) ($^{\circ}C\ d$) and the rate of leaf senescence (SR) (SPAD units $^{\circ}C\ d^{-1}$). A fifth parameter, the onset of senescence (EC90) ($^{\circ}C\ d$) was calculated. The logistic function was fitted for each plot but some trajectories lacked end tail, so $SPAD_{min}$ was unreliable and not analysed.

A senescence ideotype for a short maturity hybrid was designed to allow for the comparison of patterns of senescence as a consequence of grain yield. In the three leaf-layers and across the three seasons, leaf greenness was plotted against thermal time from a few days after flowering through to maturity. The patterns of senescence were regulated by grain yield and the ideotype was driven by either high yield under the application of fertilizer or low yield when fertilizer was not added.

As detailed in Kitonyo et al. (2018), water use was estimated as seasonal evapotranspiration, the change in soil water content between sowing and harvesting plus rainfall during the same period (French and Schultz, 1984). A correlation matrix was used to analyse the impact of evapotranspiration on the patterns of leaf senescence.

Analysis of variance (ANOVA) was conducted to assess the experimental sources of variation for all traits including parameters of fitted curves using GenStat 18th Edition (VSN International Ltd., Hertfordshire, UK) at $P \leq 0.05$ probability. Split-split plot design in GenStat was used, and tillage \times stubble \times N rate were used as the treatment structure. Treatments were assigned the main (tillage), sub-plots (stubble) and sub-sub plots (N rate), as described in our experimental design. Leaf layers were not randomly assigned in the ANOVA. Residuals for all measured variables were checked for normal distribution, and transformations were not required. For across-season or canopy layer analysis, a design in

GenStat was selected, where season (or leaf layer) \times tillage \times stubble \times N rate were set up as treatment structure. Differences between treatment means were compared and separated using Fisher's least significant difference (LSD) test at $P \leq 0.05$ (Gomez and Gomez, 1984). Relationships between variables were examined by correlations and regression analysis.

3. Results

3.1 Post-flowering growing conditions

Fig. 1 summarises post-flowering rainfall, mean daily temperature and evapotranspiration. Accumulated rainfall was 75 mm in 2015 long rains and rain events intensified as the crops aged. In 2015/2016 short rains, 50 mm was received, where larger events occurred a few days after flowering and rainfall tapered-off as crops aged. Post-flowering rainfall was evenly distributed during the 2016 long rains, which received 50 mm. There were no large within-season variations in evapotranspiration and temperature but evapotranspiration was lower during the third season. Detailed growing conditions from sowing to flowering are reported in Kitonyo et al. (2018).

3.2 Grain yield and yield components

We reported grain yield, yield components and N traits in Kitonyo et al. (2018). Briefly, grain yield varied with season and ranged from 2.3-5.3 t ha⁻¹ but tillage and stubble effects were small. N rate effects were large, and, on average, N fertilization increased grain yield by 40% compared with the unfertilized control but there were no differences in grain yield between 80 and 120 kg N ha⁻¹. Grain yield was a function of kernel number ($R^2 = 0.90$, $P < 0.001$) and kernel weight ($R^2 = 0.46$, $P < 0.001$). NRE accounted for 85% ($P < 0.001$)

variation in grain yield. NRE was proportional to NNI at flowering ($R^2 = 0.85$, $P < 0.001$). Grain protein was weakly correlated with grain yield ($R^2 = 0.26$, $P = 0.037$), and marginally with kernel weight ($R^2 = 0.11$, $P = 0.047$). Fertilized crops had 15% higher grain protein than unfertilized controls but there were no differences between 80 and 120 kg N ha⁻¹.

3.2 Time-course of leaf senescence at the whole-plant level

Fig. 2 shows the time-course of leaf senescence at the whole-plant level, and Table 1 the fitted parameters. The progression of leaf senescence followed a two-phase pattern (Eq, 1). Across seasons, season ($P < 0.001$) and N rate ($P = 0.003$) affected the rate of senescence, and the effects were larger for slope 2 compared with slope 1. Tillage ($P > 0.050$) did not affect rate of senescence while stubble amount impacted slope 1 ($P = 0.010$) but not slope 2 ($P = 0.102$). At the same scale of analysis, season ($P < 0.001$), tillage ($P < 0.001$), stubble ($P = 0.005$) altered the breakpoint between the slope 1 and slope 2 but for individual season analysis, the effects of tillage, stubble and many interactions were small. Overall, N rate effects on the breakpoint were absent ($P = 0.98$). However, when seasons were analysed separately lack of N delayed the breakpoint compared with N supply (Table 1). On average, the breakpoint was delayed by 86 °C d (~ 7 days) in the unfertilized crops compared with fertilized counterparts but there were no differences in this trait between 80 and 120 kg N ha⁻¹. This was concomitant with the fast rate of senescence in fertilized crops compared with unfertilized controls, particularly during 2016 long rains.

3.3 Time-course of leaf senescence in the canopy layers

Fig. 3, 4 and 5 show the time-course of leaf senescence at three canopy layers. N rate effects were large throughout the canopy layers (Fig. 3). The unfertilized crops had reduced

leaf greenness compared with their fertilized counterparts. However, there were no differences in leaf greenness in crops supplied with 80 and 120 kg N ha⁻¹, except for greener bottom leaves at 120 kg N ha⁻¹ compared with 80 kg N ha⁻¹ in the first season. During the third season, N rate did not alter the pattern of senescence in the middle and bottom-layer leaves.

Stubble effects were not observed in the first two seasons (Fig. 4). However, in the third season, mid-layer leaves of crops on bare ground had reduced greenness compared with crops with stubble. Tillage system did not alter leaf greenness at any of the three canopy layers (Fig. 5).

3.4 Traits of senescence

Fig. 6 shows the leaf senescence traits in canopy layers, as defined by the parameters of the logistic function (Eq. 2). Senescence patterns displayed a sequential loss of leaf greenness: first in bottom leaves, then the top leaves and eventually the leaves in the mid layer. On average, SPAD_{max} was 48 for top, 55 for mid and 38 for the bottom layers (Fig. 6 a, b, c). Tillage did not affect SPAD_{max} ($P > 0.05$) at any of the three canopy layers. Often, stubble reduced leaf greenness compared with crops on bare ground. Tillage and stubble, and interaction effects are presented in Table A1.

EC90 was not impacted by N rate, but large effects on EC50 were observed ($P < 0.001$) (Fig. 6 d, e, f). In the top and mid layers, unfertilized controls retarded EC50 compared with N fertilization. In contrast, fertilization prolonged leaf greenness in the bottom leaves compared with the unfertilized control.

Across seasons, season did not affect EC90 in the top- ($P = 0.067$) and middle-layer leaves ($P = 0.610$) but large effects were observed in the bottom leaves ($P < 0.001$). On the

other hand, season effects on EC50 were large ($P < 0.001$) in all canopy layers. In tandem with the maize senescence patterns, EC90 varied across the canopy layers ($P < 0.001$), and was neither affected by tillage ($P = 0.526$) nor N rate ($P = 0.357$) but was marginally affected by stubble ($P = 0.043$). EC90 was earlier in the bottom leaves, then the top leaves and the last to initiate senescence were the ear leaves. Similarly, EC50 varied across the canopy layers ($P < 0.001$) but unlike EC90, this trait was impacted by tillage ($P = 0.013$), N rate ($P = 0.008$) and many treatment interactions.

Rate of leaf senescence was altered by N rate ($P < 0.05$) across the canopy layers but differences were large in 2015 long rains and marginal during 2015/2016 short rains and 2016 long rains (Fig. 6 g, h, i). In fertilized crops, rate of senescence was higher in the top and mid-layer leaves, and slower in the bottom layer only when N was applied. In the unfertilized controls, rate of senescence was lower in the mid layer compared with both the top and bottom leaves, during the second and third season. There were no marked differences in the rate of senescence across the canopy layers during the first season. Overall, rate of senescence was faster in the top and mid layers (18-20 SPAD units $^{\circ}\text{C d}^{-1}$) and slower in the bottom layer (10 SPAD units $^{\circ}\text{C d}^{-1}$).

3.5 Relationship between traits of senescence and grain yield, N traits and seasonal evapotranspiration

At the whole-plant level, slope 1 and 2 and the breakpoint between the two slopes was not correlated with grain yield and yield components or N traits ($P > 0.05$).

Table 2 presents correlations between senescence parameters for canopy layers, yield components and N traits. Grain yield, yield components and N traits were strongly and positively correlated with SPAD_{max} . On the other hand, yield and N traits were weakly and

inconsistently correlated with EC90. Strong negative correlations were observed between EC50 and grain yield and NNI and NRE traits, mostly in the mid and top layer leaves. In the bottom layer, EC50 positively correlated with grain yield in two of the three seasons.

Grain yield and kernel number associated with a faster rate of senescence in the top and mid layer leaves and slower rate of senescence in the bottom leaves (Table 2). The patterns and traits of senescence and their relationship with grain yield could be summarised in a sink-driven senescence ideotype for a short maturity maize hybrid (Fig. 7). Here, SPAD_{max} and a fast rate of senescence were associated with high yield. Low grain yield was associated with prolonged leaf greenness and slow rate of senescence in the top and mid layer leaves (Fig. 7 a, b). In the bottom leaves, high yield, which was associated with N fertilization, prolonged the greenness of bottom leaves while a small sink (or shortage of N supply) induced early leaf senescence (Fig. 7 c).

In the present study, evapotranspiration did not alter the traits of senescence. In addition, crops were protected with fungicides and insecticides, thus disease and pest damage did not affect this study. Moreover, apart from varying rates of N supply, crop deficiency from other nutrients were not observed.

4. Discussion

The improvement of N economy in NT and stubble retention systems requires multiple approaches to increase N uptake, internal utilization and partitioning to the grain (Kitonyo et al., 2018). The present study demonstrates strong links between layered patterns of leaf senescence, grain yield and NRE in maize. Senescence was sink-driven and the patterns of leaf senescence modulated N fluxes from the senescing leaves to the grain. On the

other hand, sink size was dependent on N supply while the effects of tillage, stubble and many treatment interactions, and seasonal evapotranspiration were small.

4.1 Onset and progression of senescence

The pattern of leaf senescence in this study was typical, whereby loss of greenness commenced from the bottom leaves, then the top leaves, while the last remaining green leaves were the mid ones (Valentinuz and Tollenaar, 2004; Escobar-Gutiérrez and Combe, 2012). Irrespective of canopy layer, the onset of senescence (EC90) was independent of growing season and management practice, and was rarely correlated with grain yield or N traits, which is consistent with the strong genetic control of this trait (Noodén et al., 1997). On the other hand, EC50 was altered by growing conditions, whereby leaf greenness persisted during the long rain seasons but crops senesced early during the short rains season.

The progression of senescence was similar at both the whole-plant and canopy layer scales in response to N supply. At the whole plant level, the breakpoint between slope 1 and 2 occurred earlier in the fertilized crops compared with unfertilized controls. Likewise, at the canopy layer level, EC50 was delayed in the unfertilized crops compared with fertilized counterparts. This outcome could be attributed to sink strength, which regulated the rate of senescence, as illustrated in the senescence ideotype (Fig. 7), and discussed below.

4. 2 Senescence and the modulation of grain yield and N traits

The regulation of senescence by genetic and environmental factors is complex. The present study neither focussed on genetics nor comprehensively analysed the environmental effects on leaf senescence. However, the remobilization of N from the senescing organs to the

grain, which was quantified by NRE, has previously been linked with the patterns of senescence in crop plants (Masclaux-Daubresse et al., 2008; Gregersen, 2011; Wu et al., 2012). Our results showed an increase in the rate of senescence with increases in both grain yield and NRE. The amount of N remobilized increased with N supply but there were no differences between 80 and 120 kg N ha⁻¹ grain (Kitonyo et al., 2018). This suggests that the sink strength regulated the rate of senescence, and in turn NRE. In wheat, the rate of senescence increased with grain yield (Xie et al., 2016; Kitonyo et al., 2017), and a high rate of grain filling was associated with a faster rate of senescence (Xie et al., 2016).

In the source-sink transition, demand for N by the grain could have accelerated leaf senescence (Sinclair et al., 1990) but the role of crop genetics cannot be excluded. Our test variety DK8031, is an early maturity maize hybrid and has a fast dry-down syndrome. In short maturity hybrids, grain yield is often restricted by sink strength, compared with yield in long maturity hybrids (Capristo et al., 2007). In addition, short maturity hybrids have limited post-flowering N uptake from the soil, hence shortfalls in N supply may be compensated by accelerated rates of senescence and remobilization of N to the grain (Borrel et al., 2001). On the other hand, “stay-green” phenotypes have higher post-flowering N uptake and accumulate more biomass after flowering but the biomass is retained in the stems (Borrel et al., 2001).

Leaf senescence explained 42% of the variation in tropical maize grain yield under limiting N conditions (Bänziger and Lafitte, 1997). When abiotic and biotic stresses were limited, sink strength regulated the rate of senescence, in sorghum (Borrell et al., 2001), wheat (Biswas and Mandal, 1986; Xie et al., 2016) and maize (Sadras et al., 2000). Lack of grain delayed leaf senescence in maize (Wolfe et al., 1988; Antonietta et al., 2016) and sunflower (Ho and Below, 1989; Sadras et al., 2000). Other reports show accelerated leaf senescence in response to low grain yield in maize, potentially due to hormonal and nutritional signals in the regulation of senescence (Sadras et al., 2000). Enhanced

concentrations of cytokinins, for example, may delay leaf senescence in maize (He et al., 2005) but the hormone's association with reduced sink size in maize is poorly understood. Nonetheless, senescence is more nuanced in maize as it depends on hybrid (Crafts-Brandner and Poneleit, 1987). The persistence of green leaf area in the unfertilized controls in the present study could be related to reduced N fluxes to the grain due to small sink size.

The source-sink relationship in the present study could be restricted to the top and middle layer leaves but not in the bottom layer, where rate of senescence was negatively correlated with grain yield and NRE. This outcome corroborates the delay in the senescence of bottom leaves in fertilized crops compared with the unfertilized controls. This implies that the bottom leaves were less important in the remobilization of N to the grain. This observation is consistent with previous studies, where the senescence of the bottom leaves starts during the vegetative phase as the stem expands and most nutrients are exported to the roots (Pommel et al., 2006; Gregersen et al., 2013; Schippers et al., 2015). In addition to age, the early senescence of bottom leaves could be attributed to shading by the upper leaves (Maddonni et al., 2001; Borrás et al., 2003; Pommel et al., 2006). It appears that the senescence signal could have been triggered by a threshold in grain filling or a decrease in leaf N (Pommel et al., 2006).

The effects of tillage, stubble and N management practices on the patterns of leaf senescence and the associated effects on grain yield have received little attention, especially as a pathway to the improvement of NUE in maize. In wheat, NT increased SPAD_{max} compared with CT but tillage system did not affect EC90, EC50 or the rate of senescence for individual varieties (Kitonyo et al., 2017). The timing and progression of senescence not only impacts yield but also the nutritional quality of grain (Schippers et al., 2015). Despite the weak correlations between rate of senescence and grain protein in the present study, fast rates of senescence are shown to associate with high grain protein in cereals (Xie et al., 2016). A

potentially negative consequence of delayed senescence is reduced grain protein content (Masclaux-Daubresse and Chardon, 2011).

4.3 Leaf senescence ideotype

A senescence ideotype for a short maturity maize hybrid, driven by sink size, is proposed from the present study. This ideotype had high SPAD_{max} in all leaf layers, which potentially associates with higher photosynthesis (Martin et al., 2005). In the top and mid layer leaves, the onset of senescence was regulated by sink size but N fertilization delayed senescence in the bottom layer. The early onset of leaf senescence in the top and mid-layer leaves was coupled with a fast rate of senescence. While high yielding hybrids should present a delay in the onset of senescence to extend photosynthetic duration, a fast rate of senescence hastens nutrient remobilization to the grain (Wu et al., 2012). Our results contradict the delayed onset of senescence trait but high SPAD_{max} suggests the maintenance of high photosynthetic activity. Direct measurements of leaf photosynthesis are required to test this proposition.

The biochemical regulators may have strongly regulated the onset of senescence in our test hybrid (Crafts-Brandner and Poneleit, 1987). Maize derives 35-55% kernel N from post-flowering soil N uptake, while the rest is remobilised from senescing organs (Hirel et al., 2007; Gregersen et al., 2013). Increased soil N could have prolonged leaf greenness in fertilized crops compared with the unfertilized control (Rajcan and Tollenaar, 1999). Traits that promote water conservation (such as early maturity) or increase water and nutrient uptake, and high harvest index could complement the proposed senescence ideotype for a short-maturity maize hybrid (Sadras and Richards, 2014).

5. Conclusion

Understanding the factors affecting yield formation in maize is important in the management of tillage, stubble retention and N fertilization practices. In the present study, tillage and stubble retention did not affect the senescence of maize canopy. However, N was the dominant factor, and interacted with stubble in driving leaf senescence. A senescence ideotype demonstrated sink-driven patterns of leaf senescence, in which faster rates of senescence associated with N supply and high yield while slower rates of senescence were concomitant with low grain yield. Nonetheless, these patterns of senescence could be restricted to short maturity maize hybrids. Further work on a wide range of senescent and “stay-green” hybrids under contrasting tillage systems and stubble amounts, and different rates and timing of N supply is required.

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References

- Abate, T., Fisher, M., Abdoulaye, T., Kassie, G.T., Lunduka, R., Marenja, P., Asnake, W., 2017. Characteristics of maize cultivars in Africa: How modern are they and how many do smallholder farmers grow? *Agric. Food Sec.* 6, 1-17.
- Antonietta, M., Acciaresi, H., Guamet, J., 2016. Responses to N deficiency in stay green and non-stay green Argentinean hybrids of maize. *J. Agron. Crop Sci.* 202, 231-242.
- Bänziger, M., Edmeades, G., Lafitte, H., 2002. Physiological mechanisms contributing to the increased N stress tolerance of tropical maize selected for drought tolerance. *Field Crops Res.* 75, 223-233.
- Bänziger, M., Lafitte, H., 1997. Efficiency of secondary traits for improving maize for low-nitrogen target environments. *Crop Sci.* 37, 1110-1117.
- Barracough, P.B., Lopez-Bellido, R., Hawkesford, M.J., 2014. Genotypic variation in the uptake, partitioning and remobilisation of nitrogen during grain-filling in wheat. *Field Crops Res.* 156, 242-248.
- Beyene, Y., Gowda, M., Suresh, L., Mugo, S., Olsen, M., Oikeh, S.O., Juma, C., Tarekegne, A., Prasanna, B.M., 2017. Genetic analysis of tropical maize inbred lines for resistance to maize lethal necrosis disease. *Euphytica* 213 (9), 224.
- Biswas, A., Mandal, S., 1986. Monocarpic senescence in wheat: Influence of sterile glumes and ear. *Physiol. Plant.* 67, 431-434.
- Bonada, M., Sadras, V.O., Fuentes, S., 2013. Effect of elevated temperature on the onset and rate of mesocarp cell death in berries of Shiraz and Chardonnay and its relationship with berry shrivel. *Aust. J. Grape Wine Res.* 19, 87-94.
- Bonhomme, R., 2000. Bases and limits to using 'degree. day' units. *Eur. J. Agron.* 3, 1-10.
- Borrás, L., Maddonni, G., Otegui, M., 2003. Leaf senescence in maize hybrids: plant population, row spacing and kernel set effects. *Field Crops Res.* 82, 13-26.

496 Borrel, A., Hammer, G., E, O., 2001. Stay-green: A consequence of the balance between
 497 supply and demand for nitrogen during grain filling? *Ann. Appl. Biol.* 138, 91-95.

498 Borrell, A., Hammer, G., Oosterom, E., 2001. Stay-green: A consequence of the balance
 499 between supply and demand for nitrogen during grain filling? *Ann. Appl. Biol.* 138,
 500 91-95.

501 Brouder, S.M., Gomez-Macpherson, H., 2014. The impact of conservation agriculture on
 502 smallholder agricultural yields: A scoping review of the evidence. *Agric. Ecosyst.*
 503 *Environ.* 187, 11-32.

504 Capristo, P.R., Rizzalli, R.H., Andrade, F.H., 2007. Ecophysiological yield components of
 505 maize hybrids with contrasting maturity. *Agron. J.* 99, 1111-1118.

506 Christopher, J.T., Veyradier, M., Borrell, A.K., Harvey, G., Fletcher, S., Chenu, K., 2014.
 507 Phenotyping novel stay-green traits to capture genetic variation in senescence
 508 dynamics. *Funct. Plant Biol.* 41, 1035-1048.

509 Crafts-Brandner, S.J., Poneleit, C.G., 1987. Effect of ear removal on CO₂ exchange and
 510 activities of ribulose biphosphate carboxylase/oxygenase and phosphoenolpyruvate
 511 carboxylase of maize hybrids and inbred lines. *Plant Physiol.* 84, 261-265.

512 Davies, P., Gan, S., 2012. Towards an integrated view of monocarpic plant senescence. *Russ.*
 513 *J. Plant Physiol.* 59, 467-478.

514 Dobermann, A., 2007. Nutrient use efficiency – measurement and management. In: Krauss,
 515 A., Isherwood, K., Heffer, P. (Eds.), *Fertilizer best management practices general*
 516 *principles, strategy for their adoption and voluntary initiatives vs regulations.*
 517 *International Fertilizer Industry Association, Paris, France, pp. 1-28.*

518 Escobar-Gutiérrez, A.J., Combe, L., 2012. Senescence in field-grown maize: from flowering
 519 to harvest. *Field Crops Res.* 134, 47-58.

520 FAO, 2015. What is conservation agriculture? <http://www.fao.org/ag/ca/1a.html>.

521 Feller, U., Anders, I., Mae, T., 2007. Rubiscolytics: fate of Rubisco after its enzymatic
522 function in a cell is terminated. *J. Exp. Bot.* 59, 1615-1624.

523 French, R., Schultz, J., 1984. Water use efficiency of wheat in a Mediterranean-type
524 environment. I. The relation between yield, water use and climate. *Crop Pasture Sci.*
525 35, 743-764.

526 Gastal, F., Lemaire, G., Durand, J.-L., Louarn, G., 2015. Quantifying crop responses to
527 nitrogen and avenues to improve nitrogen-use efficiency. In: Sadras, V.O., Calderini,
528 D.F. (Eds), *Crop physiology - Applications for genetic improvement and agronomy*,
529 2nd edn. Academic press, Elsevier, London. 161-206.

530 Gomez, K.A., Gomez, A.A., 1984. Statistical procedures for agricultural research. John
531 Wiley & Sons, New York.

532 Gregersen, P.L., 2011. Senescence and nutrient remobilization in crop plants. In:
533 Hawkesford, M.J., Barraclough, P. (Eds), *The molecular and physiological basis of*
534 *nutrient use efficiency in crops*. 1st edn. Wiley Blackwell, UK. pp. 83-102.

535 Gregersen, P.L., Culetic, A., Boschian, L., Krupinska, K., 2013. Plant senescence and crop
536 productivity. *Plant Mol. Biol.* 82, 603-622.

537 Harding, S.A., Guikema, J.A., Paulsen, G.M., 1990. Photosynthetic decline from high
538 temperature stress during maturation of wheat. *Plant Physiol.* 92, 648-653.

539 He, P., Osaki, M., Takebe, M., Shinano, T., Wasaki, J., 2005. Endogenous hormones and
540 expression of senescence-related genes in different senescent types of maize. *J. Exp.*
541 *Bot.* 56, 1117-1128.

542 Hirel, B., Le Gouis, J., Ney, B., Gallais, A., 2007. The challenge of improving nitrogen use
543 efficiency in crop plants: towards a more central role for genetic variability and
544 quantitative genetics within integrated approaches. *J. Exp. Bot.* 58, 2369-2387.

545 Ho, I., Below, F.E., 1989. Whole plant senescence of sunflower following seedhead removal.
 546 Plant physiol. 91, 85-90.

547 Jaetzold, R., Schmidt, H., Hornetz, B., Shisanya, C.A., 2006. Farm management handbook of
 548 Kenya. Natural conditions and farm information. Ministry of Agriculture/GTZ,
 549 Nairobi, Kenya.

550 Justes, E., Mary, B., Meynard, J.-M., Machet, J.-M., Thelier-Huche, L., 1994. Determination
 551 of a critical nitrogen dilution curve for winter wheat crops. Ann. Bot. 74, 397-407.

552 Kitonyo, O.M., Sadras, V.O., Zhou, Y., Denton, M.D., 2017. Evaluation of historic
 553 Australian wheat varieties reveals increased grain yield and changes in senescence
 554 patterns but limited adaptation to tillage systems. Field Crops Res. 206, 65-73.

555 Kitonyo, O.M., Sadras, V.O., Zhou, Y., Denton, M.D., 2018. Nitrogen fertilization modifies
 556 maize yield response to tillage and stubble in a sub-humid tropical environment. Field
 557 Crops Res. 223, 113-124.

558 Lim, P.O., Kim, H.J., Gil Nam, H., 2007. Leaf senescence. Annu. Rev. Plant Biol. 58, 115-
 559 136.

560 Liu, F., Jensen, C.R., Andersen, M.N., 2005. A review of drought adaptation in crop plants:
 561 changes in vegetative and reproductive physiology induced by ABA-based chemical
 562 signals. Aust. J. Agric. Res. 56, 1245-1252.

563 Maddonni, G., Otegui, M.E., Cirilo, A.G., 2001. Plant population density, row spacing and
 564 hybrid effects on maize canopy architecture and light attenuation. Field Crops Res.
 565 71, 183-193.

566 Martin, A., Belastegui-Macadam, X., Quilleré, I., Floriot, M., Valadier, M.H., Pommel, B.,
 567 Andrieu, B., Donnison, I., Hirel, B., 2005. Nitrogen management and senescence in
 568 two maize hybrids differing in the persistence of leaf greenness: agronomic,
 569 physiological and molecular aspects. New Phytol. 167, 483-492.

570 Masclaux-Daubresse, C., Chardon, F., 2011. Exploring nitrogen remobilization for seed
 571 filling using natural variation in *Arabidopsis thaliana*. *J. Exp. Bot.* 62, 2131-2142.
 572 Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L.,
 573 Suzuki, A., 2010. Nitrogen uptake, assimilation and remobilization in plants:
 574 challenges for sustainable and productive agriculture. *Ann. Bot.* 105, 1141-1157.
 575 Masclaux-Daubresse, C., Reisdorf-Cren, M., Orsel, M., 2008. Leaf nitrogen remobilisation
 576 for plant development and grain filling. *Plant Biol.* 10, 23-36.
 577 Monsanto, 2017. DK8031, the hybrid for food security. <http://www.monsantoafrica.com>
 578 Noodén, L.D., Guiamét, J.J., John, I., 1997. Senescence mechanisms. *Physiol. Plant.* 101,
 579 746-753.
 580 Plénet, D., Lemaire, G., 1999. Relationships between dynamics of nitrogen uptake and dry
 581 matter accumulation in maize crops. Determination of critical N concentration. *Plant*
 582 *Soil* 216, 65-82.
 583 Pommel, B., Gallais, A., Coque, M., Quillere, I., Hirel, B., Prioul, J., Andrieu, B., Floriot, M.,
 584 2006. Carbon and nitrogen allocation and grain filling in three maize hybrids differing
 585 in leaf senescence. *Eur. J. Agron.* 24, 203-211.
 586 Rajcan, I., Tollenaar, M., 1999. Source: sink ratio and leaf senescence in maize: I. Dry matter
 587 accumulation and partitioning during grain filling. *Field Crops Res.* 60, 245-253.
 588 Sadras, V., Echarte, L., Andrade, F., 2000. Profiles of leaf senescence during reproductive
 589 growth of sunflower and maize. *Ann. Bot.* 85, 187-195.
 590 Sadras, V.O., Richards, R.A., 2014. Improvement of crop yield in dry environments:
 591 benchmarks, levels of organisation and the role of nitrogen. *J. Exp. Bot.* 65, 1981-
 592 1995.

593 Schippers, J.H., Jing, H.-C., Hille, J., Dijkwel, P.P., 2007. Developmental and hormonal
 594 control of leaf senescence. In: Gan, S. (Ed), Senescence processes in plants. 1st Edn.
 595 Blackwell publishing, New York, USA. pp. 145-170.

596 Schippers, J.H., Schmidt, R., Wagstaff, C., Jing, H.-C., 2015. Living to die and dying to live:
 597 the survival strategy behind leaf senescence. *Plant physiol.* 169, 914-930.

598 Sinclair, T., Bennett, J., Muchow, R., 1990. Relative sensitivity of grain yield and biomass
 599 accumulation to drought in field-grown maize. *Crop Sci.* 30, 690-693.

600 Staswick, P.E., 1992. Jasmonate, genes, and fragrant signals. *Plant Physiol.* 99, 804-807.

601 Thomas, H., Ougham, H., 2015. Senescence and crop performance. In: Sadras, V.O.,
 602 Calderini, D.F. (Eds.), *Crop physiology: Applications for genetic improvement and*
 603 *agronomy.* Elsevier, London, pp. 223-249.

604 Valentinuz, O.R., Tollenaar, M., 2004. Vertical profile of leaf senescence during the grain-
 605 filling period in older and newer maize hybrids. *Crop Sci.* 44, 827-834.

606 van Doorn, W.G., Woltering, E.J., 2004. Senescence and programmed cell death: substance
 607 or semantics? *J. Exp. Bot.* 55, 2147-2153.

608 Verhulst, N., Govaerts, B., Nelissen, V., Sayre, K.D., Crossa, J., Raes, D., Deckers, J., 2011.
 609 The effect of tillage, crop rotation and residue management on maize and wheat
 610 growth and development evaluated with an optical sensor. *Field Crops Res.* 120, 58-
 611 67.

612 Wei, H., Meng, T., Li, X., Dai, Q., Zhang, H., Yin, X., 2018. Sink-source relationship during
 613 rice grain filling is associated with grain nitrogen concentration. *Field Crops Res.* 215,
 614 23-38.

615 Wilkinson, S., Davies, W.J., 2002. ABA-based chemical signalling: the co-ordination of
 616 responses to stress in plants. *Plant Cell Environ.* 25, 195-210.

617 Wolfe, D., Henderson, D., Hsiao, T., Alvino, A., 1988. Interactive water and nitrogen effects
618 on senescence of maize. I. Leaf area duration, nitrogen distribution, and yield. *Agron.*
619 *J.* 80, 859-864.

620 Wu, X.Y., Kuai, B.K., Jia, J.Z., Jing, H.C., 2012. Regulation of Leaf Senescence and Crop
621 Genetic Improvement. *J. Integr. Plant Biol.* 54, 936-952.

622 Xie, Q., Mayes, S., Sparkes, D.L., 2016. Early anthesis and delayed but fast leaf senescence
623 contribute to individual grain dry matter and water accumulation in wheat. *Field*
624 *Crops Res.* 187, 24-34.

625 Yang, J., Udvardi, M., 2017. Senescence and nitrogen use efficiency in perennial grasses for
626 forage and biofuel production. *J. Exp. Bot.* 69, 855-865.

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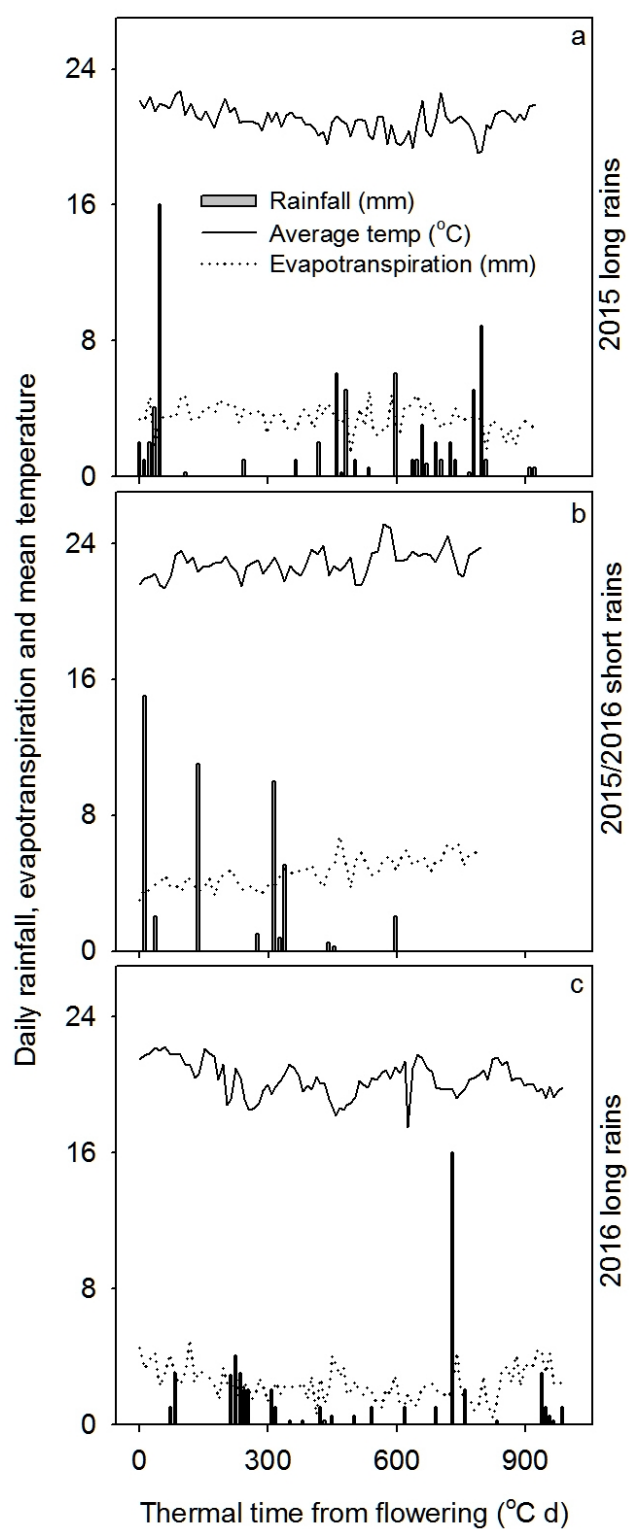


Fig. 1. Growing conditions from flowering (50% pollen shedding) to harvesting of maize during 2015 long rains (a), 2015/2016 short rains (b) and 2016 long rains (c) at the Kenya Agricultural and Livestock Research Organisation, Embu research station.

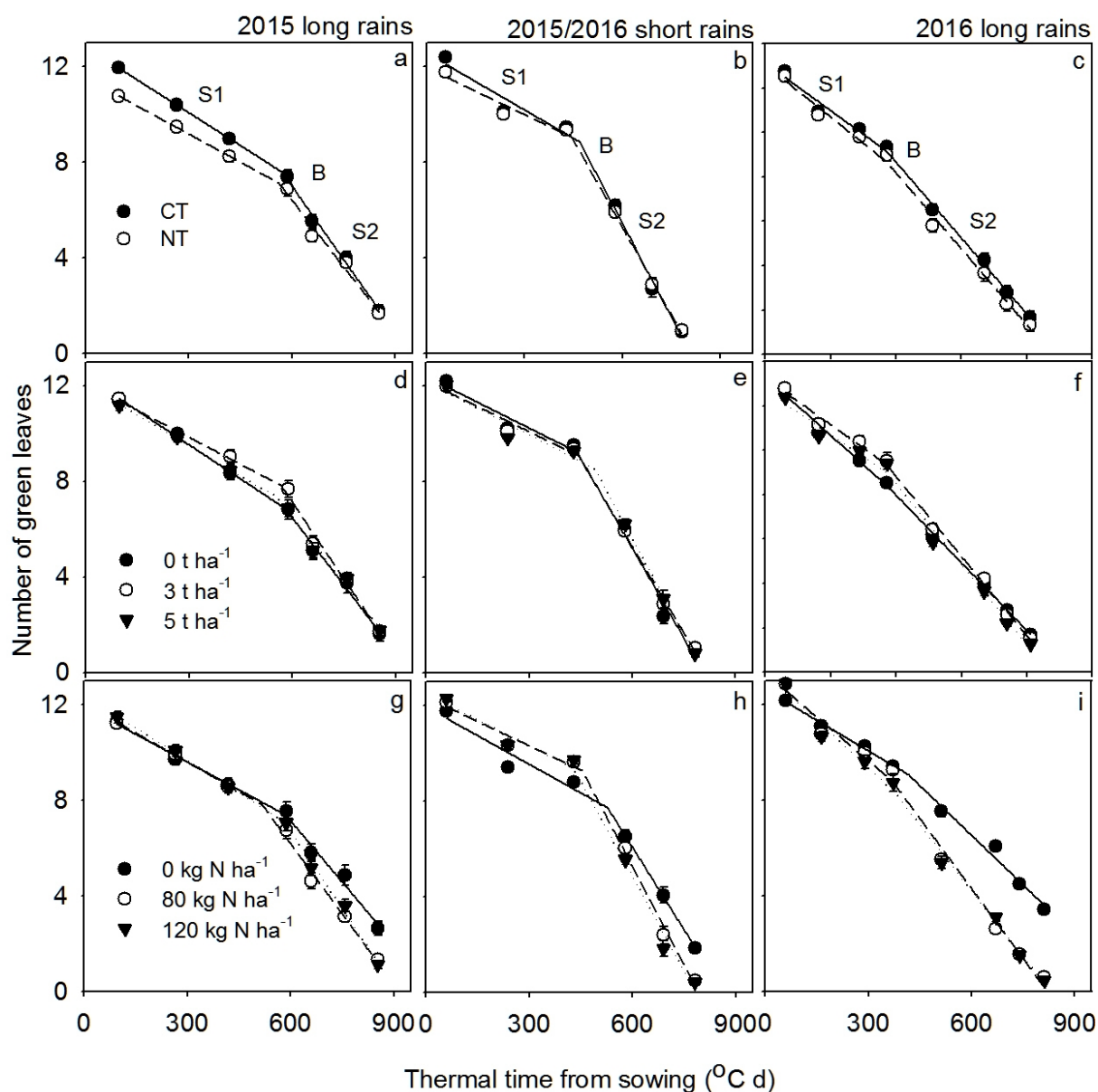


Fig. 2. Time-course of leaf senescence of maize grown under conventional tillage (CT) and no-till (NT) (a-c), three amounts of stubble (d-f) and three N rates (g-i) during the 2015 long rains (a, d, g), 2015/2016 short rains (b, e, h) and 2016 long rains (c, f, i) at Kenya Agricultural and Livestock Research Organisation, Embu research station. Slope S1 and S2 are the rate of senescence. The breakpoint and slopes are summarised in Table 1.

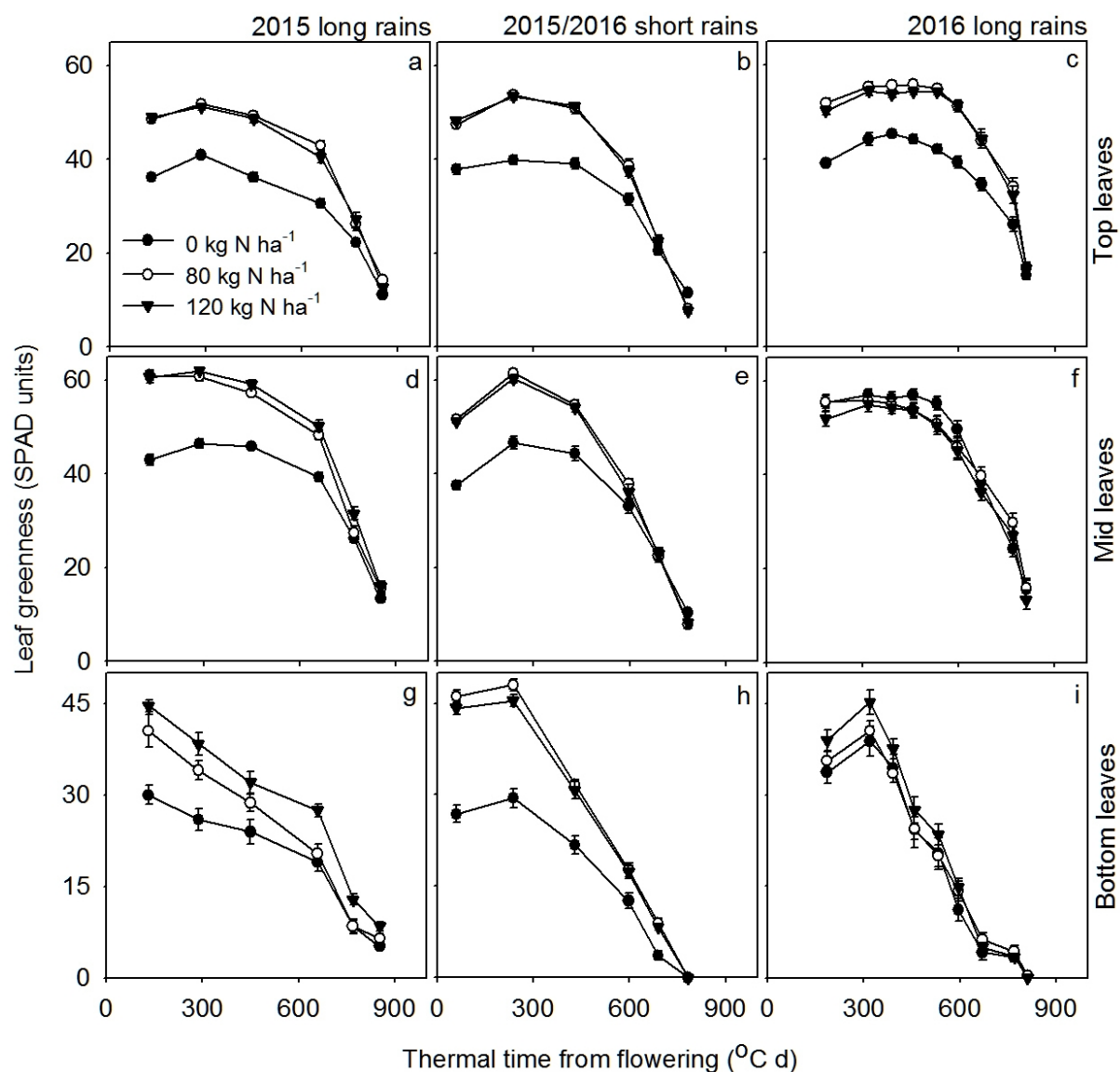


Fig. 3. The effect of N rate on leaf senescence of maize at three canopy layers during 2015 long rains (a, d, g), 2015/2016 short rains (b, e, h) and 2016 long rains (c, f, i) at the Kenya Agricultural and Livestock Research Organisation, Embu research station. Error bars are 1 standard error of the mean.

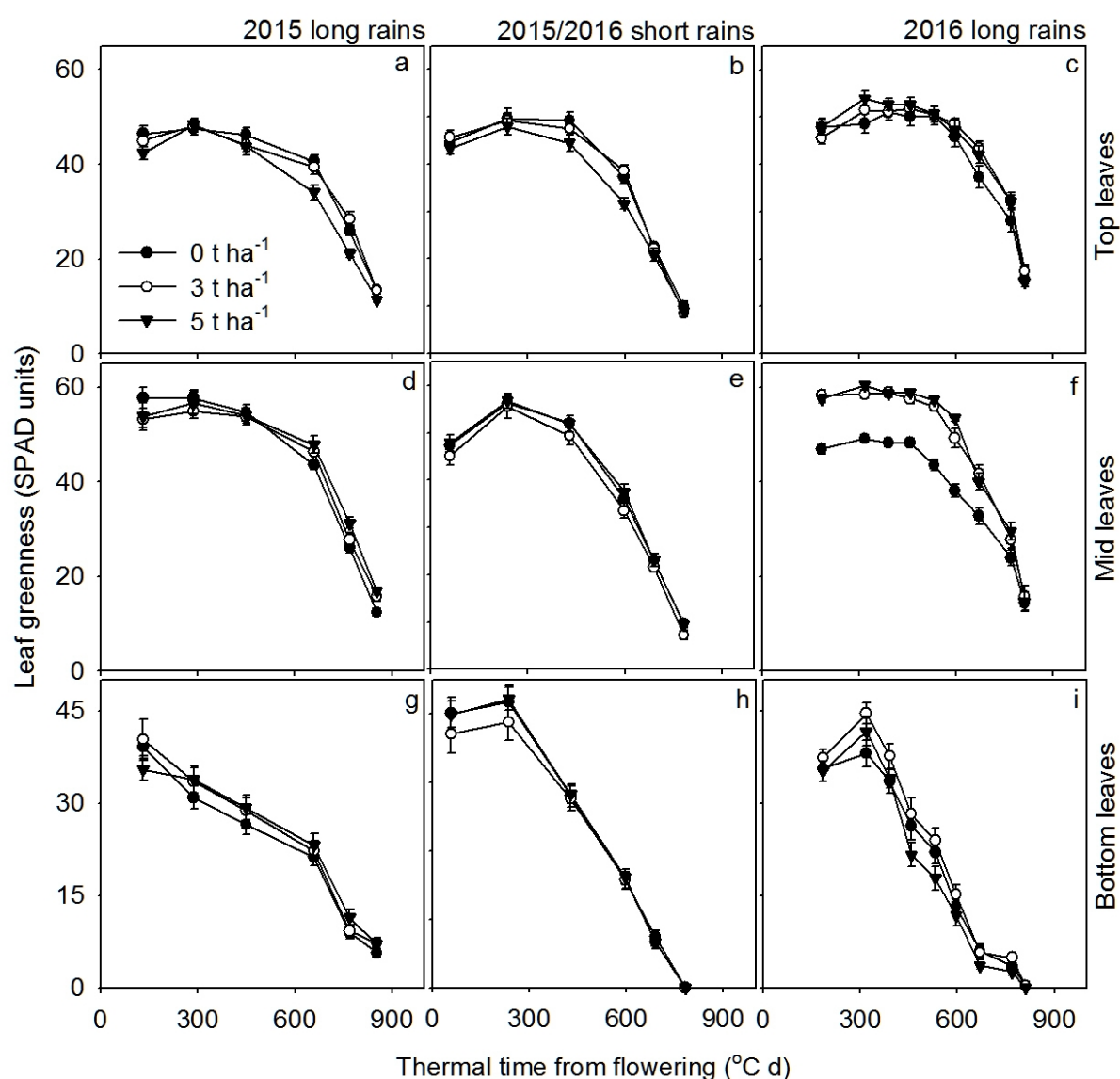


Fig. 4. The effect of stubble amount on leaf senescence of maize at three canopy layers during 2015 long rains (a, d, g), 2015/2016 short rains (b, e, h) and 2016 long rains (c, f, i) at the Kenya Agricultural and Livestock Research Organisation, Embu research station. Error bars are 1 standard error of the mean.

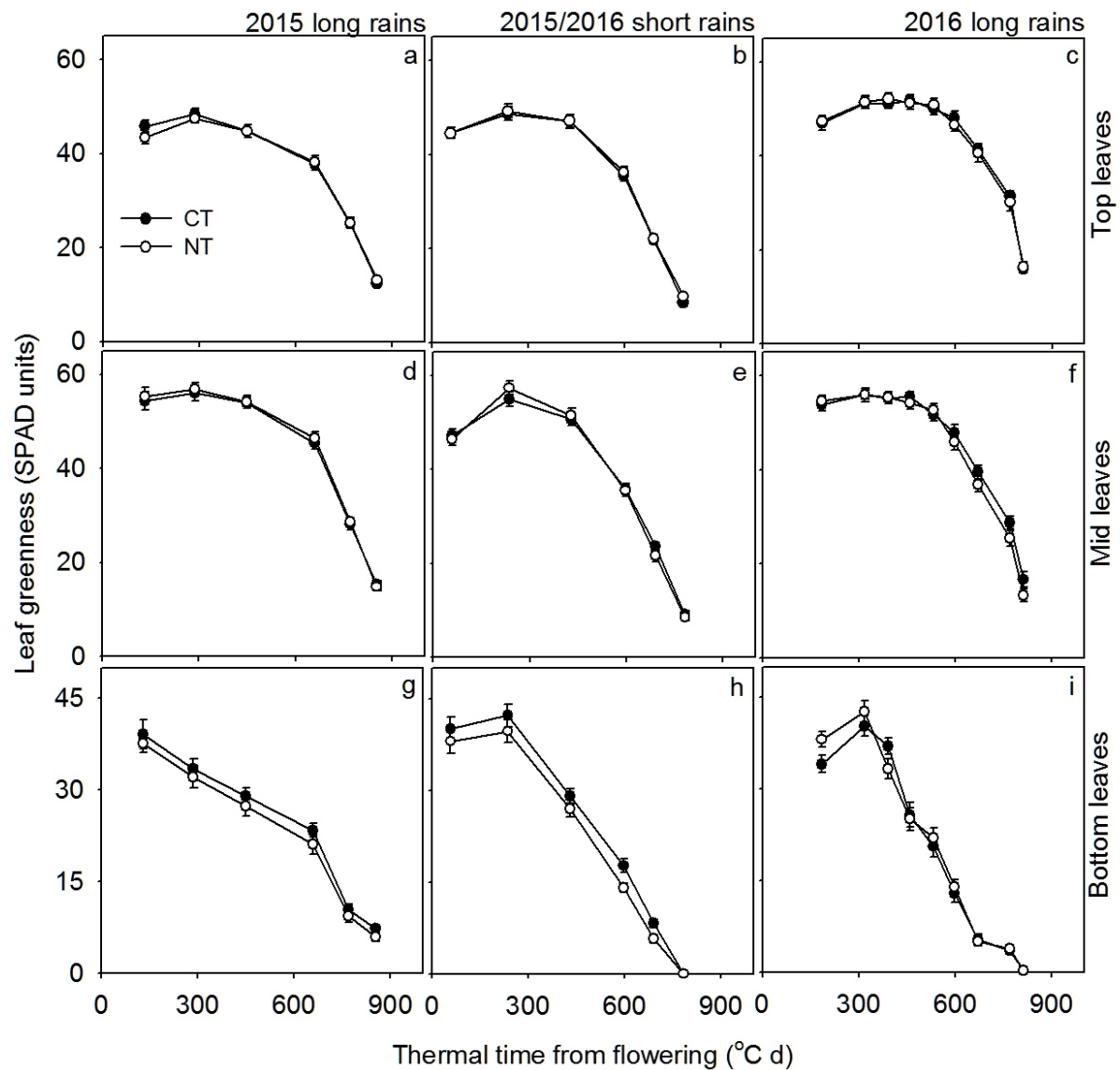


Fig. 5. The effect of tillage system on leaf senescence of maize at three canopy layers during 2015 long rains (a, d, g), 2015/2016 short rains (b, h, h) and 2016 long rains (c, f, i) at the Kenya Agricultural and Livestock Research Organisation, Embu research station. Error bars are 1 standard error of the mean.

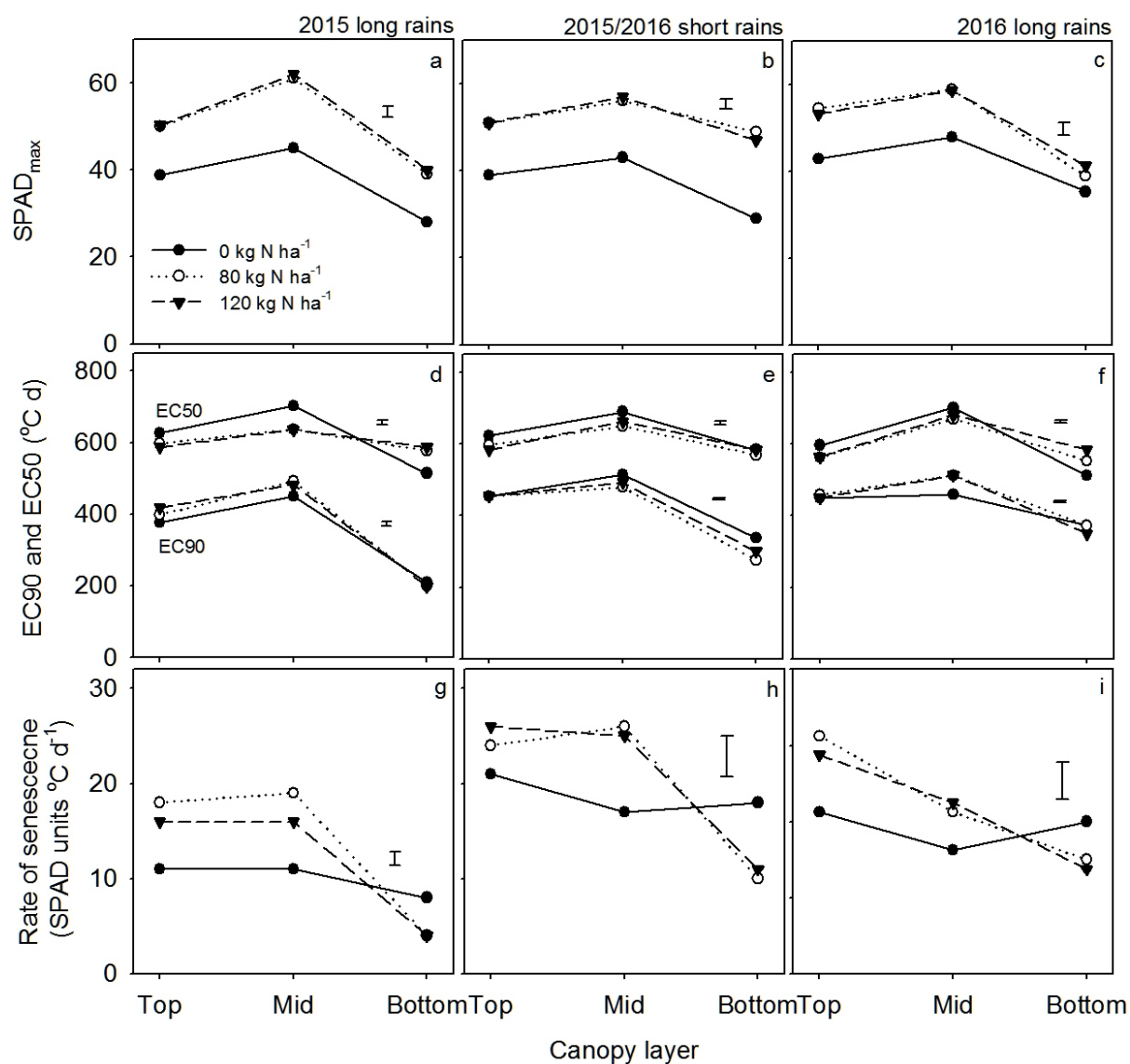


Fig 6. Nitrogen-driven traits of leaf senescence at different layers of maize canopy grown at the Kenya Agricultural and Livestock Research Organization, Embu research station during 2015 long rains (a, d, g), 2015/2016 short rains (b, e, h) and 2016 long rains. Vertical bars are LSD at 5% probability.

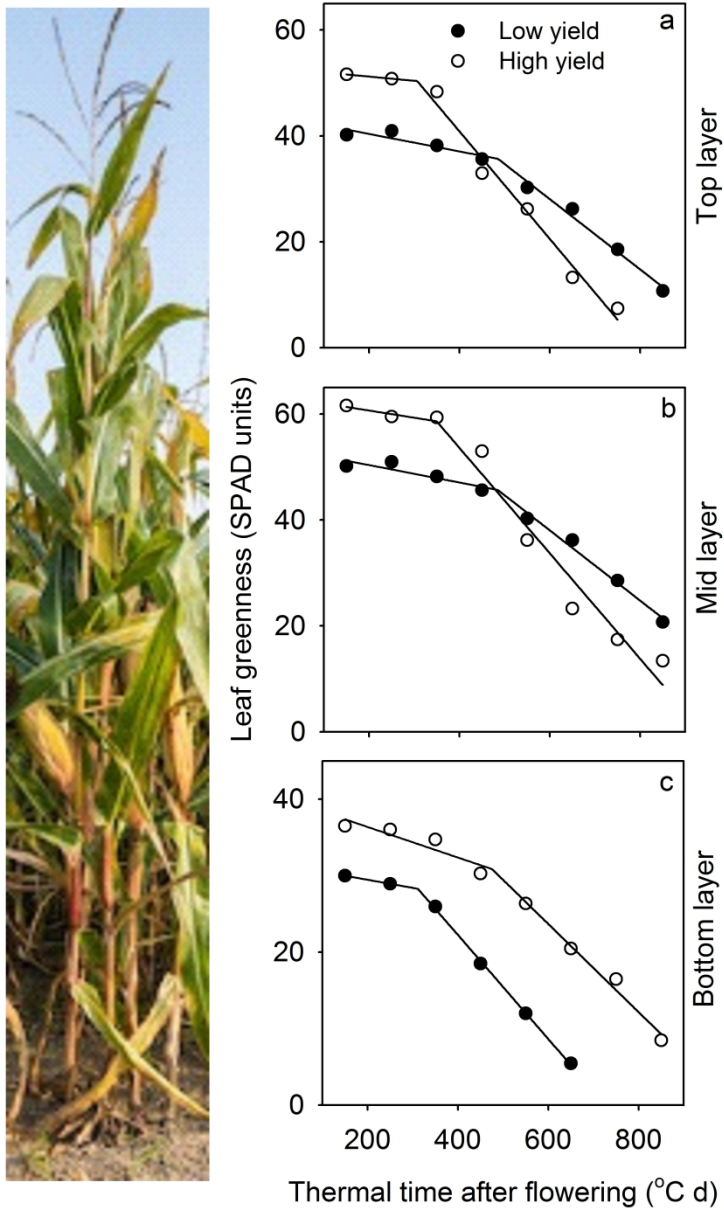


Fig. 7. Sink-driven leaf senescence ideotype of a short maturity maize hybrid. Data points are actual SPAD units across three seasons. High yield is grain yield with the addition of fertilizer N while low yield is grain yield without the addition of N. The photo is maize at dough stage (R4) when kernels are half filled with solid starch.

Table 1. Traits of leaf senescence at the whole-plant level of maize grown under conventional tillage (CT) and no-till (NT), three amounts of stubble and three N rates during 2015 long rains, 2015/2016 short rains and 2016 long rains at Kenya Agricultural and Livestock Research Organisation, Embu research station. Slopes 1 and 2 are the rate of senescence (number of green leaves °C d⁻¹) and the breakpoint (°C d) is the transition between the two slopes. The breakpoint and slopes were estimated by fitting Eq. 1.

Treatment	2015 long rains			2015/2016 short rains			2016 long rains		
	Breakpoint	Slope 1	Slope 2	Breakpoint	Slope 1	Slope 2	Breakpoint	Slope 1	Slope 2
Tillage									
CT	571a	-0.009a	-0.022a	480a	-0.008a	-0.028a	434a	-0.011a	-0.018a
NT	570a	-0.007a	-0.020a	472a	-0.007a	-0.027a	391a	-0.011a	-0.018a
Stubble									
0 t ha ⁻¹	575a	-0.009a	-0.019a	449a	-0.007a	-0.026a	430a	-0.013a	-0.016b
3 t ha ⁻¹	580a	-0.007a	-0.023a	482a	-0.007a	-0.028a	403a	-0.010b	-0.019a
5 t ha ⁻¹	557a	-0.009a	-0.020a	498a	-0.008a	-0.029a	405a	-0.010b	-0.019a
N rate									
0 kg N ha ⁻¹	614a	-0.008a	-0.019b	526a	-0.008a	-0.027a	491a	-0.009b	-0.016b
80 kg N ha ⁻¹	527c	-0.008a	-0.022a	442b	-0.007a	-0.027a	355b	-0.012a	-0.020a
120 kg N ha ⁻¹	570b	-0.009a	-0.022a	460b	-0.007a	-0.028a	392b	-0.013a	-0.019a
Interactions ANOVA									
Tillage x stubble	ns	ns	ns	ns	ns	ns	ns	**	ns
Tillage x N rate	ns	ns	ns	ns	ns	ns	ns	***	**
Stubble x N rate	**	ns	***	ns	ns	ns	ns	***	*
Tillage x stubble x N rate	**	ns	*	ns	ns	ns	ns	*	ns

Within a column, means followed by the same letter are not significantly different at 0.05 probability. *P < 0.05, **P < 0.01 and ***P < 0.001; ns: not significant.

Table 2. Correlation coefficients between senescence traits and grain yield, yield components and N traits in three canopy layers of maize grown under conventional tillage and no-till, three amounts of stubble and three N rates during 2015 long rains, 2015/2016 short rains and 2016 long rains at the Kenya Agricultural and Livestock Research Organisation, Embu research station.

Traits	2015 long rains				2015/2016 short rains				2016 long rains			
	SPAD _{max}	EC90	EC50	SR	SPAD _{max}	EC90	EC50	SR	SPAD _{max}	EC90	EC50	SR
Top layer												
Grain yield	0.83	0.29	-0.50	0.40	0.81	0.05	-0.45	0.21	0.86	0.06	-0.54	0.31
Kernel number	0.70	0.35	-0.50	0.45	0.70	0.05	-0.40	0.13	0.87	0.07	-0.54	0.34
Kernel weight	0.40	-0.08	-0.07	-0.08	0.46	0.12	-0.26	0.29	0.14	-0.05	-0.11	-0.04
Grain protein					0.46	-0.10	-0.33	0.08	0.64	0.12	-0.39	0.36
NNI	0.68	0.18	-0.52	0.24	0.84	0.14	-0.45	0.38				
NRE	0.81	0.25	-0.58	0.31	0.80	0.14	-0.34	0.23				
N uptake	-0.17	-0.02	0.15	-0.02	-0.05	0.03	-0.03	0.15	0.17	-0.18	-0.12	-0.10
NAE	-0.02	0.21	-0.06	0.37	-0.35	-0.24	0.27	-0.32	-0.09	0.06	0.01	0.12
Mid layer												
Grain yield	0.73	0.32	-0.52	0.54	0.85	-0.38	-0.32	0.63	0.82	0.24	-0.55	0.31
Kernel number	0.68	0.17	-0.63	0.48	0.64	-0.31	-0.25	0.55	0.84	0.26	-0.55	0.30
Kernel weight	0.26	0.06	-0.08	0.14	0.71	-0.21	-0.28	0.40	0.09	-0.15	-0.07	0.01
Grain protein					0.60	-0.15	-0.27	0.30	0.69	0.15	-0.27	0.22
NNI	0.68	0.00	-0.58	0.27	0.83	-0.42	-0.36	0.66				
NRE	0.82	0.11	-0.62	0.40	0.77	-0.47	-0.26	0.51				
N uptake	-0.33	0.01	0.17	-0.02	0.07	0.18	-0.15	0.04	0.02	-0.15	0.15	-0.12
NAE	-0.33	0.32	0.20	0.35	-0.31	0.07	-0.02	-0.41	0.11	-0.12	-0.27	-0.05
Bottom layer												
Grain yield	0.70	-0.01	0.74	-0.50	0.82	-0.03	-0.44	-0.53	0.33	-0.01	0.42	-0.49
Kernel number	0.62	-0.03	0.68	-0.43	0.64	-0.08	-0.43	-0.54	0.33	-0.06	0.42	-0.48
Kernel weight	0.24	0.15	0.34	-0.22	0.64	0.05	-0.16	-0.26	0.07	0.12	0.04	-0.08
Grain protein					0.45	-0.11	-0.14	-0.21	0.35	0.01	0.36	-0.30
NNI	0.64	-0.06	0.66	-0.52	0.78	0.03	-0.34	-0.51				
NRE	0.67	-0.15	0.77	-0.61	0.72	-0.03	-0.33	-0.43				
N uptake	-0.19	-0.20	-0.35	0.12	-0.06	0.09	-0.23	-0.06	0.09	-0.32	-0.26	0.01
NAE	0.18	0.03	-0.02	0.26	0.12	-0.25	-0.02	-0.13	-0.14	0.16	0.09	0.01

SPAD_{max}, maximum SPAD; EC90, onset of senescence; EC50, loss of 50% maximum SPAD; SR, rate of senescence; NNI, nitrogen nutrition index at flowering; NRE, nitrogen remobilisation efficiency; NAE, nitrogen agronomic efficiency; Increasing colour intensity indicates P value for positive (blue) and negative (red) correlations; uncoloured coefficients had P > 0.05. n = 54.

Colour legend		P < 0.05	
		P < 0.01	
		P < 0.001	

Appendix

Table A1. Tillage and stubble-driven traits of leaf senescence at different canopy layers of maize grown at the Kenya Agricultural and Livestock Research Organization, Embu research station during 2015 long rains, 2015/2016 short rains and 2016 long rains.

Treatment	2015 long rains				2015/2016 short rains				2016 long rains			
	SPAD _{max}	EC90	EC50	SR	SPAD _{max}	EC90	EC50	SR	SPAD _{max}	EC90	EC50	SR
Top leaves												
Tillage												
CT	47.0a	398a	597a	14a	47a	454a	610a	23a	49.9a	450a	574a	21a
NT	45.8b	398a	607a	15a	47a	454a	597a	25a	50.5a	458a	574a	23a
Stubble												
0 t ha ⁻¹	47.5a	418a	607a	17a	48a	466a	597a	28a	49.3b	450a	574a	22a
3 t ha ⁻¹	45.8a	408a	597a	15a	48a	466a	610a	25a	49.8b	466a	574a	27a
5 t ha ⁻¹	45.9a	376a	607a	13a	46a	429a	623a	18b	51.5a	450a	584a	18a
Interactions ANOVA												
Tillage × stubble	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Tillage × N rate	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns
Stubble × N rate	ns	ns	ns	ns	ns	ns	ns	ns	***	ns	ns	ns
Tillage × stubble × N rate	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Mid leaves												
Tillage												
CT	56b	482a	669a	16a	52a	492a	690a	23a	55.4a	493a	670a	16a
NT	57a	460a	659a	15a	52a	503a	663a	22a	54.8a	502a	681a	17a
Stubble												
0 t ha ⁻¹	58a	460a	659a	15a	53a	503a	663a	23a	56.1a	542a	681a	20a
3 t ha ⁻¹	54b	482a	669a	17a	51a	492a	677a	23a	53.8a	548b	681a	15b
5 t ha ⁻¹	55b	471a	659a	15a	53a	503a	677a	21a	55.5a	496b	681a	14b
Interactions ANOVA												
Tillage × stubble	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Tillage × N rate	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Stubble × N rate	**	ns	ns	ns	***	ns	ns	ns	ns	**	ns	ns
Tillage × stubble × N rate	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*
Bottom leaves												
Tillage												
CT	36a	210a	567a	6a	43a	228a	597a	13a	37.5b	381a	563a	13a
NT	36a	186a	556a	5a	40b	314a	569a	13a	40.2a	350a	542a	13a
Stubble												
0 t ha ⁻¹	37a	199a	556a	5a	43a	228a	584a	12a	37.7a	372a	584a	12a
3 t ha ⁻¹	37a	199a	556a	5a	39b	314a	597a	14a	39.7a	361a	553a	14a
5 t ha ⁻¹	34a	222a	567a	6a	42b	300a	569a	12a	39.1a	350a	522b	14a
Interactions ANOVA												
Tillage × stubble	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Tillage × N rate	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
Stubble × N rate	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
Tillage × stubble × N rate	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Within a column, means followed by the same letter are not significantly different at 0.05 probability. *P < 0.05, **P < 0.01 and ***P < 0.001; ns: not significant.

**Nitrogen supply and sink demand modify the patterns of leaf senescence in the canopy
of maize in a sub-humid tropical environment**

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14 Highlights

- 15 • Leaf senescence in maize was studied under combinations of tillage, stubble, N rate
- 16 and seasons
- 17 • Nitrogen ~~supply~~ altered leaf the patterns of senescence~~e~~
- 18 • but sSeason, stubble and tillage effects were small ~~did not affect the patterns of~~
- 19 ~~senescence~~
- 20 • Sink size regulated senescence in the maize canopy
- 21 • A senescence ideotype is proposed

22

23

Abstract

Senescence is a key physiological process that can regulate crop grain yield. Patterns of leaf senescence and its association with grain yield for a short maturity maize hybrid were investigated in a factorial ~~experiment combining~~ combination of two tillage treatments (conventional and no-till), three amounts of stubble (0, 3 and 5 t ha⁻¹) and three N rates (0, 80 and 120 kg N ha⁻¹) over three seasons in 2015 long rains, 2015/2016 short rains and 2016 long rains. Leaf senescence from flowering to harvest was assessed at (a) the whole-plant scale by the visual scoring of dry leaves and (b) the canopy-layer scale by measuring leaf greenness with a SPAD 502 chlorophyll meter. A bilinear model was used to quantify the patterns of senescence at the whole-plant scale. A logistic function was fitted to estimate the traits of senescence at three canopy layers (top, mid, bottom), including minimum and maximum SPAD, onset of senescence (EC90), time to loss of 50% maximum SPAD (EC50) and the rate of senescence in each layer. Nitrogen rate effect on patterns and traits of senescence were large and its interactions with stubble were more frequent than interactions between other treatments. Tillage and stubble amount had marginal effects. EC50 was delayed in the unfertilized controls compared with fertilized crops and was negatively correlated with grain yield. Rate of senescence was faster in fertilised crops compared with unfertilized controls at both whole-plant and canopy-layer levels. Grain yield, grain number and nitrogen remobilization efficiency were associated with faster rates of senescence in the top and mid leaves but with slower rates of senescence in the bottom layer leaves. We advance a sink-driven leaf senescence ideotype for high yield and efficient use of nitrogen for short maturity maize.

46 **Keywords:** ~~conservation agriculture~~ no-till, stubble, source-sink, N remobilization effeciency,
47 SPAD

48

49 **Abbreviations:** NT, no-till; CT, conventional tillage; N, nitrogen; NNI, N nutrition index; NRE,
50 N remobilization efficiency; NUE, N use efficiency; °C, degree celsius; °C d, growing degree
51 day; t, tonnes; ha, hectare; P, phosphorus; kg, kilogram; g, grams; L, litre; DK, DeKalb; DM, dry
52 mass; SPAD, chlorophyll unit; SPAD_{min}, minimum SPAD unit before harvesting; SPAD_{max},
53 maximum SPAD unit before the onset of senescence; EC90, onset of senescence; EC50, time to
54 loss of 50% SPAD_{max}.

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1. Introduction

No-till (NT) management and stubble retention protects the soil from water and soil erosion, improves soil water capture and storage, and promotes soil chemical and physical properties, which leads to higher yields ~~Conservation agriculture, which encompasses reduced soil disturbance or no-till, permanent soil cover with stubble mulch or cover crops and crop rotations or intercropping (FAO, 2015), seeks to arrest soil degradation and improve crop production (FAO, 2015).~~ Previous ~~s~~Studies on the impact of these practices on crops and cropping systems ~~have primarily been~~are restricted to crop growth and yield, water and nutrient economy, with little emphasis on physiological processes (Verhulst et al., 2011; Brouder and Gomez-Macpherson, 2014). Kitonyo et al. (2018). ~~(under review)~~ ~~demonstrated~~concludes that nitrogen (N) ~~modified~~modifies crop response to ~~no-till~~NT and stubble retention in a sub-humid tropical environment, by altering N nutrition index (NNI), which explained most of the variation in crop growth rate in the critical window of yield determination. Crop growth rate in turn accounted for most of the variation in kernel number and yield while ~~by altering N nutrition index (NNI) and crop growth rate during the critical window of yield determination. In addition,~~ grain yield was ~~proportional to~~linearly correlated with N remobilization efficiency (NRE), which is defined as the difference in shoot N at flowering and harvesting (Kitonyo et al., ~~2018~~under review). The manipulation of patterns of leaf senescence ~~which that~~ impact photosynthesis, harvest index and N remobilization, and potentially N use efficiency has received little attention in ~~conservation agriculture~~cropping systems (Masclaux-Daubresse et al., 2010; Wu et al., 2012).

In monocarpic plants such as maize, leaf senescence is a developmental process that involves the gradual loss of green leaf area in the older leaves and finally the whole plant (Gregersen et al., 2013). High yield potential in maize has been achieved through the extension of photosynthetic duration and increased harvest index, two traits that are related

with leaf senescence (Bänziger et al., 2002; [Wu et al., 2012](#)). Genetic and environmental factors trigger and regulate senescence (Gregersen et al., 2013; Thomas and Ougham, 2015). There is considerable genetic variation in the patterns of senescence in maize (Gregersen et al., 2013). Broadly, senescent hybrids senesce earlier irrespective of growing conditions while the “stay-green” phenotypes show prolonged green leaf area duration (Gregersen et al., 2013).

The genetic control of senescence is complex and involves both programmed cell death and hormonal regulation (Wilkinson and Davies, 2002; van Doorn and Woltering, 2004; Liu et al., 2005; Lim et al., 2007). Hormonal signals such as abscisic acid, ethylene, cytokinins and jasmonic acid regulate senescence in response to stress and source-sink ratios (Harding et al., 1990; Staswick, 1992; Schippers et al., 2007; Davies and Gan, 2012). Environmental factors like water, nutrient stress and temperature modulate senescence (Gregersen, 2011). Both leaf area duration and green leaf area proportionally impact grain yield (Gregersen et al., 2013). The ratio of assimilate supply (i.e., source) to demand (i.e., sink) during grain filling also regulates senescence, and impacts nutrient fluxes from the senescing leaves to the grain (Feller et al., 2007; Wei et al., 2018). In some species, ~~for~~ [examples such as](#) tomato, low source:sink ratios [favour](#)s senescence but in maize the response of senescence to source:sink ratios [varies](#) with hybrid (Crafts-Brandner and Ponleit, 1987; Sadras et al., 2000).

The translocation of N from senescing tissues to the grain indirectly impacts N use efficiency (NUE) (Masclaux-Daubresse et al., 2008; Gregersen, 2011). At the crop level, NUE, the ratio between grain yield and fertilizer supplied (Dobermann, 2007) depends on N uptake from the soil, internal utilization and the subsequent partitioning and remobilization of N to the grain (Masclaux-Daubresse et al., 2010). Nitrogen remobilisation is fundamental for crop N economy since it controls a large part of N fluxes from sources to sinks

(Masclaux-Daubresse et al., 2008). N remobilization efficiency (NRE) accounted for 85% variation in grain yield in both maize (Kitonyo et al., ~~under review~~2018) and wheat (Barraclough et al., 2014). Despite the fundamental role played by N remobilization in crop N economy (Yang and Udvardi, 2017), patterns of leaf senescence ~~have been~~are least explored for the improvement of NUE in ~~conservation agriculture~~NT and stubble retention systems.

In the model advanced by Christopher et al. (2014), the key traits of senescence are quantified: the minimum leaf greenness before harvesting, maximum greenness before the onset of senescence, the timing of onset of senescence, and the progression and rate of senescence. Profiles of leaf senescence vary within the spatial arrangement of leaves, which affects light interception and attenuation, thus shaded leaves often senesce earlier than unshaded ones (Maddonni et al., 2001). In addition, carbon assimilation and N transfer from senescing leaves to the grain varies with leaf position (Feller et al., 2007). The middle leaves and top leaves ~~are a source of~~provide N for ~~maize~~-grain filling in maize, while the bottom leaves export more N to the roots than to the grain (Feller et al., 2007).

This study investigated ~~maize the patterns of~~ leaf senescence ~~patterns in maize~~ and their association with yield in a context of ~~no-till~~NT ~~and~~ and stubble retention. Our aims were to (i) characterize the time-course of post-flowering leaf senescence ~~under during grain filling~~ ~~under~~ conventional tillage and ~~no-till~~NT, ~~and~~ with three stubble and three N rates, and (ii) establish relationships between senescence and grain yield, yield components, crop N status quantified as NNI, and traits related to nitrogen use efficiency.

2. Materials and methods

The experiments are fully described in Kitonyo et al. (~~under review~~2018). Here we briefly summarise treatments and experimental design, and focus on measurements and analysis of senescence.

2.1 Site

A field study was conducted at the Kenya Agricultural and Livestock Research Organisation, Embu research station (0.515 °S, 37.273 °E, 1425 masl), for three consecutive seasons during the 2015 long rains, 2015/2016 short rains and 2016 long rains. –Embu is in the upper midland zone three (UM3) and has a sub-humid climate with mean annual temperatures of range between 18–24 °C (Jaetzold et al., 2006). In this environment, there are two rainy five-month rainy seasons; ~~the short rains season from October to February and~~ the long rains that occur between April and August, and the short rains season from October to February. Soils are deep (> 2.5 m) well-weathered humic nitisols with low exchangeable bases and relatively high P-sorption, and of medium to low fertility (Jaetzold et al., 2006).

2.2 Treatments and experiment design

Effects of conventional tillage (CT) and no-till (NT), three amounts of maize stubble (0, 3 and 5 t ha⁻¹) and three fertilizer N rates (0, 80 and 120 kg N ha⁻¹) were evaluated under continuous maize cropping over three seasons during 2015 long rains, 2015/2016 short rains and 2016 long rains. Tillage and stubble treatments were applied two weeks before sowing in the same plot in all seasons. Prescribed amounts of stubble were supplied in the first season while in the next two seasons, additional stubble allowed for undecomposed material. In CT plots stubble was chopped to small pieces and incorporated into the soil by digging to 15 cm

depth while the NT treatments were not disturbed and stubble was surface applied without cutting. Fertilizer N was sourced from urea (46% N) and side-banded on the sowing rows in the fractions of $\frac{1}{3}$ at sowing and $\frac{2}{3}$ at six-leaf stage. All plots received 60 P kg ha⁻¹ of basal fertilizer from triple super phosphate that was side banded on the sowing rows.

Experiments were laid out as split-split plot design with three replications. Tillage systems formed the main plots, stubble amount the sub-plots and N rate formed the sub-sub plots. The main plots measured 31 m long ~~and~~ 12.25 m wide, ~~28 m x 3.75 m for~~ sub-plots were 28 m x 3.75 m while the sub-sub plots were 7 m x 3.75 m. In all plots, sowing was done manually by opening 5 cm deep holes with a sharp machete to hold seed and fertilizer.

A locally adapted short-maturity DeKalb (DK) 8031 maize hybrid was used. DK_8031 has a fast dry-down syndrome and has a yield potential of 5-8 t ha⁻¹, and is frequently used as a commercial check in breeding studies in Kenya -(Abate et al., 2017; Beyene et al., 2017). (Monsanto, 2017). Crops were sown at the onset of rains: on 25th March for 2015 long rains season, 19th October for 2015/2016 short rains season and 3rd April for 2016 long rains season. Crops were sown with 0.75 m between rows and 0.25 ~~between plants~~ within plants a row to give an approximate density of 5.3 plants m⁻². Weeds were controlled with Roundup® (glyphosate) before sowing and Dual Gold® (960 g L⁻¹ S-metolachlor) after sowing in both tillage systems while in-crop weeds were removed by hoe-weeding in CT plots but with Twiga 2, 4-D® (Dimethylamine salt and 2, 4-diclorophenoxy acetic acid) in NT plots. Insect pests were controlled with Thunder® (100 g L⁻¹ imidacloprid) at 300 mL ha⁻¹ (Kitonyo et al., 2018).

2.3 Measurements

A phenological scale (CIMMYT, 2017) was used to determine flowering as 50% shedding of pollen. Growing degree days (°C d) were computed as the average mean day temperature minus 10 °C, which is the base temperature for maize- (Bonhomme, 2000)in our environment. Timing of maize maturity was determined by dislodging kernels and observing the development of a black layer at the point of kernel attachment to the cob.

Five plants per plot were tagged for the ~~visual~~ assessment of leaf senescence at the whole-plant scale. Senescence was scored visually from a few days after flowering through to maturity by recording leaves ~~which~~ that presented more than 50% green leaf area. At the same phenological range, vertical profiles of leaf greenness were measured from two tagged plants per plot. From top to bottom leaves, leaf greenness was measured using SPAD 502 chlorophyll meter (Konica Minolta, Japan), at several points of the whole-leaf length and the mean SPAD unit was recorded. Leaf counts and SPAD measurements were sampled at least every two weeks. Even though rare for DK8031, in the circumstances where a plant produced two ears, the lower ear was removed.

~~Kitonyo et al. (under review)~~ Kitonyo et al. (2018) described yield collection and computations for traits related to N use efficiency. Briefly grain yield was measured from a harvest area of 14.6 m², and kernel number m⁻² and 1000 kernel weight were determined in all seasons. Grain protein was measured by near infra-red spectroscopy using FOSS Infratec® 1241 grain analyser during 2015/2016 short rains and 2016 long rains only. Nitrogen nutrition index at NNI ~~at~~ flowering was calculated as the ratio between the actual N concentration of the ~~above-ground~~ shoot biomass and the critical N concentration at the same crop mass (Gastal et al., 2015). Critical N concentration (N_c), the minimum N concentration required to maximise biomass, was computed as: $N_c = 3.4 * (DM)^{-0.37}$; where DM is the

actual crop biomass in t ha⁻¹ (Justes et al., 1994; Plénet and Lemaire, 1999). N remobilization efficiency (NRE) was calculated as (N content at flowering – N content at maturity)/N content at flowering.

2.4 Analysis of senescence

At the whole-plant level, rate of senescence was quantified by fitting a bilinear model of leaf counts against thermal time from flowering using the piece-wise routine of SigmaPlot version 10.0 (Systat Software, Inc., San Jose California USA, www.systatsoftware.com) (Eq. 1). This model was used to describe monocarp senescence in fruit (Bonada et al., 2013), and estimated slope 1 (Eq. 1a), slope 2 (Eq. 1b) and a breakpoint thermal time at the transition from the first to the second slope.

$$\text{Slope 1} = \frac{y_1(T - t) + y_2(t - t_1)}{T - t_1} \quad \text{For, } T > t_1 \quad (1a)$$

$$\text{Slope 2} = \frac{y_2(t_1 - t) + y_3(t - T)}{t_1 - T} \quad \text{For, } T < t_1 \quad (1b)$$

In Eq. 1, y_1 is number of green leaves at the onset of senescence, y_2 is the number of green leaves at harvesting, y_3 is the number of green leaves at breakpoint, T is the breakpoint (°C d), t is onset of senescence (°C d) and t_1 is the end of senescence (°C d). The bilinear model was fitted for each plot.

The profiles of leaf greenness were separated into three canopy layers: the mid layer of which comprised the ear leaf, one leaf below the ear leaf and two leaves above the ear leaf. The top and the bottom layers consisted of the leaves above and below the mid layer, respectively. A logistic regression function was adopted to fit a time-course of leaf senescence to quantify the traits of senescence at each canopy layer using SigmaPlot (Christopher et al., 2014; Kitonyo et al., 2017) (Eq. 2).

$$y = SPAD_{min} + \frac{SPAD_{max} - SPAD_{min}}{1 + \left(\frac{x}{EC50}\right)^{SR}} \quad (2)$$

Eq. 2 estimated four parameters, including the minimum SPAD before harvesting ($SPAD_{min}$), maximum SPAD before the onset of senescence ($SPAD_{max}$), time to loss of 50% of $SPAD_{max}$ ($EC50$) ($^{\circ}C$ d) and the rate of leaf senescence (SR) ($SPAD$ units $^{\circ}C$ d $^{-1}$). A fifth parameter, the onset of senescence ($EC90$) ($^{\circ}C$ d) was calculated. The logistic function was fitted for each plot but some trajectories lacked end tail, so $SPAD_{min}$ was unreliable and not analysed.

A senescence ideotype for a short maturity hybrid was designed to allow for the comparison of patterns of senescence as a consequence of grain yield. In the three leaf-layers and across the three seasons, leaf greenness was plotted against thermal time from a few days after flowering through to maturity. The patterns of senescence were regulated by grain yield and the ideotype was driven by either high yield under the application of fertilizer or low yield when fertilizer was not added.

As detailed in Kitonyo et al. (2018), water use was estimated as ~~the~~ seasonal evapotranspiration, the change in soil water content between sowing and harvesting plus rainfall during the same period (French and Schultz, 1984). A correlation matrix was used to analyse the impact of evapotranspiration on the patterns of leaf senescence.

Analysis of variance (ANOVA) was conducted to assess the experimental sources of variation for all traits including parameters of fitted curves using GenStat 18th Edition (VSN International Ltd., Hertfordshire, UK)-~~SAS 9.2 for windows~~, at $P \leq 0.05$ probability. Split-split plot design in GenStat was used, and tillage \times stubble \times N rate were used as the treatment structure. Treatments were assigned the main (tillage), sub-plots (stubble) and sub-sub plots (N rate), as described in our experimental design. Leaf layers were not randomly assigned in the ANOVA. Residuals for all measured variables were checked for normal distribution, and transformations were not required. For across-season or canopy layer analysis, a design in GenStat was selected, where season (or leaf layer) \times tillage \times stubble \times N rate were set up as treatment structure. Differences between treatment means were compared and separated using Fisher's least significant difference (LSD) test at $P \leq 0.05$ (Gomez and Gomez, 1984). Relationships between variables were examined by correlations and regression analysis.

3. Results

3.1 Post-flowering growing conditions

Fig. 1 summarises post-flowering rainfall, mean daily temperature and evapotranspiration. Accumulated rainfall was 75 mm in 2015 long rains and rain the rainfall events intensified as the crops aged. In 2015/2016 short rains, 50 mm was received, where; the largermajority of events occurred a few days after flowering and rainfall tapered-off as

crops aged. Post-flowering rainfall was evenly distributed during the 2016 long rains, which received 50 mm. There were no large within-season variations in evapotranspiration and temperature but evapotranspiration was lower during the third season. Detailed growing conditions from sowing to flowering are reported in ~~Kitonyo et al. (under review)~~Kitonyo et al. (2018).

3.2 Grain yield and yield components

We reported grain yield, yield components and N traits in ~~Kitonyo et al. (under review)~~Kitonyo et al. (2018). Briefly, grain yield varied with season and ranged from 2.3-5.3 t ha⁻¹ but, tillage and stubble effects were small, N rate effects were large, and, on average, N fertilization increased grain yield by 40% compared with the unfertilized control but there were no differences in grain yield between 80 and 120 kg N ha⁻¹. Grain yield was a function of kernel number ($R^2 = 0.90$, $P < 0.001$) and kernel weight ($R^2 = 0.46$, $P < 0.001$). NRE accounted for 85% ($P < 0.001$) variation in grain yield. NRE was proportional to NNI at flowering ($R^2 = 0.85$, $P < 0.001$). Grain protein was weakly correlated with grain yield ($R^2 = 0.26$, $P = 0.037$), and marginally with kernel weight ($R^2 = 0.11$, $P = 0.047$). Fertilized crops had 15% higher grain protein than unfertilized controls but there were no differences between 80 and 120 kg N ha⁻¹.

3.2 Time-course of leaf senescence at the whole-plant level

Fig. 2 shows the time-course of leaf senescence at the whole-plant level and Table 1 the fitted parameters. The progression of leaf senescence followed a two-phase pattern (Eq. 1). Across seasons, S_{season} ($P < 0.001$) and N rate ($P = 0.003$) affected the rate of senescence, and the effects were larger for slope 2 than compared with slope 1. Tillage ($P >$

0.050) did not affect rate of senescence while stubble amount ~~had effects impacted on~~ slope 1 ~~only~~ ($P = 0.010$) ~~but not slope 2 ($P = 0.102$)~~. ~~At the same scale of analysis, s~~Season ($P < 0.001$), tillage ($P < 0.001$), stubble ($P = 0.005$) altered the breakpoint between the ~~two~~ ~~slopes~~ slope 1 and slope 2 but for individual season analysis, the effects of tillage, stubble and many interactions were small. ~~In the overall analysis,~~ N rate effects on the breakpoint were absent (~~but N rate~~ ($P = 0.98$)). ~~However, when seasons were analysed separately lack of N delayed the breakpoint compared with N supply effects were not observed. However, ANOVA for individual seasons showed that the breakpoint was mostly altered by N rate while tillage, stubble and interaction effects were small~~ (Table 1). On average, the breakpoint was delayed by 86 °C d (~ 7 days) in the unfertilized crops compared with fertilized counterparts but there were no differences in this trait between 80 and 120 kg N ha⁻¹. This was concomitant with the fast rate of senescence in fertilized crops compared with unfertilized controls, particularly during 2016 long rains.

3.3 Time-course of leaf senescence in the canopy layers

Fig. 3, 4 and 5 show the time-course of leaf senescence at three canopy layers. N rate effects were large throughout the canopy layers (Fig. 3). The unfertilized crops had reduced leaf greenness compared with their fertilized counterparts. However, there were no differences in leaf greenness in crops supplied with 80 and 120 kg N ha⁻¹, except for greener bottom leaves at 120 kg N ha⁻¹ compared with 80 kg N ha⁻¹ in the first season. During the third season, N rate did not alter the pattern of senescence ~~of in~~ the middle and bottom ~~layer~~ leaves.

Stubble effects were not observed in the first two seasons (Fig. 4). ~~During~~ However, in the third season, mid-layer leaves of crops on bare ground had reduced greenness

compared with crops with stubble. Tillage system did not alter leaf greenness at any of the three canopy layers (Fig. 5).

3.4 Traits of senescence

Fig. 6 shows the leaf senescence traits in canopy layers, as defined by the parameters of the logistic function (Eq. 2). Senescence patterns displayed a sequential loss of leaf greenness: first in bottom leaves, then the top leaves and eventually the leaves in the mid layer. On average, $SPAD_{max}$ was 48 for top, 55 for mid and 38 for the bottom layers (Fig. 6 a, b, c). Tillage did not affect $SPAD_{max}$ ($P > 0.05$) at any of the three canopy layers. Often, stubble reduced leaf greenness compared with crops on bare ground. Tillage and stubble, and interaction effects are presented in Table A1.

EC90 was not impacted by N rate, but large effects on EC50 were observed ($P < 0.001$) (Fig. 6 d, e, f). In the top and mid layers, unfertilized controls retarded EC50 compared with N fertilization. In contrast, fertilization prolonged leaf greenness in the bottom leaves compared with the unfertilized control.

Across seasons, ~~results for EC90 and EC50 were the most interesting (not shown).~~ Season did not affect EC90 in the top (P = 0.067) and middle-layer leaves (P = 0.610) but large effects were observed in the bottom ~~layer-leaves~~ (P < 0.001). On the other hand, season effects on EC50 were large (P < 0.001) in all canopy layers. In tandem with the maize senescence patterns, EC90 varied across the canopy layers (P < 0.001), and was neither affected by tillage (P = 0.526) nor N rate (P = 0.357) but was marginally affected by stubble (P = 0.043). EC90 was earlier in the bottom leaves, then the top leaves and the last to initiate senescence were the ear leaves. Similarly, EC50 varied across the canopy layers (P < 0.001)

but unlike EC90, this trait was impacted by tillage ($P = 0.013$), N rate ($P = 0.008$) and many treatment interactions.

Rate of leaf senescence was altered by N rate ($P < 0.05$) across the canopy layers but differences were large in 2015 long rains and marginal during 2015/2016 short rains and 2016 long rains (Fig. 6 g, h, i). In fertilized crops, rate of senescence was higher in the top and mid-layer leaves, and slower in the bottom layer only when N was applied. In the unfertilized controls, rate of senescence was lower in the mid layer compared with both the top and bottom leaves, during the second and third season. There were no marked differences in the rate of senescence across the canopy layers during the first season. Overall, rate of senescence was faster in the top and mid layers (18-20 SPAD units $^{\circ}\text{C d}^{-1}$) and slower in the bottom layer (10 SPAD units $^{\circ}\text{C d}^{-1}$).

3.5 Relationship between traits of senescence and grain yield, ~~yield components and~~ N traits and seasonal evapotranspiration

At the whole-plant level, Slope-slope 1 and 2 and the breakpoint between the two slopes did-was not correlated with grain yield and yield components or N traits ($P > 0.05$).

Table 2 presents correlations between senescence parameters for canopy layers, yield components and N traits. Grain yield, yield components and N traits were strongly and positively correlated with SPAD_{max} . On the other hand, yYield and N traits were weakly and inconsistently correlated with EC90. Strong negative correlations were observed between EC50 and grain yield and NNI and NRE traits, mostly in the mid and top layer leaves. In the bottom layer, EC50 positively correlated with grain yield in two of the three seasons.

Grain yield and kernel number associated with a faster rate of senescence in the top and mid layer leaves and slower rate of senescence in the bottom leaves (Table 2).

The patterns and traits of senescence and their relationship with grain yield could be summarised in a sink-driven senescence ideotype for a short maturity maize hybrid (Fig. 7). Here, SPAD_{max} and a fast rate of senescence were associated with high yield. Low grain yield was associated with prolonged leaf greenness and slow rate of senescence in the top and mid layer leaves (Fig. 7 a, b). In the bottom leaves, high yield, which was associated with N fertilization, prolonged the greenness of bottom of bottom-leaves while a small sink (or shortage of N supply) induced early leaf senescence (Fig. 7 c).

~~In Kitonyo et al. (2018), tillage system, stubble amount and N rate did not affect seasonal evapotranspiration while in the present study, evapotranspiration did not alter the traits of senescence. In addition, crops were protected with fungicides and insecticides, thus disease and pest damage did not affect this study. Moreover, apart from varying rates of N supply, crop deficiency from other nutrients were not observed.~~

4. Discussion

The improvement of N economy in ~~conservation agriculture~~NT and stubble retention systems requires multiple approaches to increase N uptake, internal utilization and partitioning to the grain ((Kitonyo et al., ~~under review~~2018). The present study demonstrates strong links between layered patterns of leaf senescence, grain yield and NRE in maize. Senescence was sink-driven and the patterns of leaf senescence modulated N fluxes from the senescing leaves to the grain. ~~In addition~~On the other hand, sink size was dependent on N supply ~~but while~~ the effects of tillage, stubble and many treatment interactions, ~~and seasonal evapotranspiration~~ -were small.

4.1 Onset and progression of senescence

The pattern of leaf senescence in this study was typical, whereby loss of greenness commenced from the bottom leaves, then the top leaves, ~~an~~ while the last remaining green leaves were the mid ones (Valentinuz and Tollenaar, 2004; Escobar-Gutiérrez and Combe, 2012). Irrespective of canopy layer, the onset of senescence (EC90) was independent of growing season and management practice, and ~~was seldom~~ ~~hardly~~ rarely correlated with grain yield or N traits, ~~which is~~ consistent with the strong genetic control of this trait (Noodén et al., 1997). On the other hand, EC50 was altered by growing conditions, whereby leaf greenness persisted during the long rain seasons but crops senesced early during the short rains season.

The progression of senescence was similar at both the whole-plant and canopy layer scales in response to N supply. At the whole plant level, the breakpoint between slope 1 and 2 occurred earlier in the fertilized crops compared with unfertilized controls. Likewise, at the canopy layer level, EC50 was delayed in the unfertilized crops compared with fertilized counterparts. This outcome could be attributed to sink strength, which regulated the rate of senescence, as illustrated in the senescence ideotype (Fig. 7), and discussed below.

4. 2 Senescence and the modulation of grain yield and N traits

The regulation of senescence by genetic and environmental factors is complex. The present study neither focussed on genetics nor comprehensively analysed the environmental effects on leaf senescence. However, the remobilization of N from the senescing organs to the grain, which was quantified by NRE, has ~~consistently~~ ~~previously~~ been linked with the patterns of senescence in crop plants (Masclaux-Daubresse et al., 2008; Gregersen, 2011; Wu et al., 2012). Our results showed an increase in the rate of senescence with increases in both grain yield and NRE. The amount of N remobilized increased with N supply but there were

no differences between 80 and 120 kg N ha⁻¹ grain (Kitonyo et al., ~~under review~~2018). This suggests that the sink strength regulated the rate of senescence, and in turn NRE. In wheat, the rate of senescence increased with grain yield (Xie et al., 2016; Kitonyo et al., 2017), and a high rate of grain filling ~~was~~ associated with a faster rate of senescence (Xie et al., 2016).

In the source-sink transition, demand for N by the grain could have accelerated the leaf senescence (Sinclair et al., 1990) but the role of crop genetics ~~should cannot~~ be excluded. ~~We used~~Our test variety DK8031, ~~is~~ an early maturity maize hybrid ~~which and~~ has a fast dry-down syndrome. ~~(Monsanto, 2017)~~. In short maturity hybrids, grain yield is often restricted by sink strength, compared with yield in long maturity hybrids (Capristo et al., 2007). In addition, short maturity hybrids have limited post-flowering N uptake from the soil, hence shortfalls in N supply may be compensated by accelerated rates of senescence and remobilization of N to the grain (Borrel et al., 2001). On the other hand, “stay-green” phenotypes have higher post-flowering N uptake and accumulate more biomass after flowering but the biomass is retained in the stems (Borrel et al., 2001).

Leaf senescence explained 42% of the variation in tropical maize grain yield under limiting N conditions (Bänziger and Lafitte, 1997). When abiotic and biotic stresses ~~are were~~ limited, sink strength ~~has been shown to regulate~~d the rate of senescence, in sorghum (Borrell et al., 2001), wheat (Biswas and Mandal, 1986; Xie et al., 2016) and maize (Sadras et al., 2000). Lack of grain delayed leaf senescence in maize (Wolfe et al., 1988; Antonietta et al., 2016) and sunflower (Ho and Below, 1989; Sadras et al., 2000). Other reports showed ~~accelerated leaf senescence in response to that~~ low grain yield ~~accelerated leaf senescence~~ in maize. ~~(Sadras et al., 2000)~~potentially due to hormonal and nutritional signals in the regulation of senescence (Sadras et al., 2000). ~~Enhanced contents~~concentrations of cytokinins, for example, may delay leaf senescence in maize (He et al., 2005) but the hormone’s association with reduced sink size in maize is poorly understood.

~~However~~Nonetheless, senescence is more nuanced in maize as it depends on hybrid (Crafts-Brandner and Poneleit, 1987). The persistence of green leaf area in the unfertilized controls in the present study could be related to reduced N fluxes to the grain due to small sink size.

The source-sink relationship in the present study could be restricted to the top and middle layer leaves but not in the bottom layer, where rate of senescence was negatively correlated with grain yield and NRE. This outcome corroborates the delay in the senescence of bottom leaves in fertilized crops compared with the unfertilized controls. This ~~implied~~ implies that the bottom leaves were less important in the remobilization of N to the grain. This observation is consistent with previous studies, where the senescence of the bottom leaves starts during the vegetative phase as the stem expands and most nutrients are exported to the roots (Pommel et al., 2006; Gregersen et al., 2013; Schippers et al., 2015). In addition to age, the early senescence of bottom leaves could be ~~attributable~~ attributed to shading by the upper leaves (Maddonni et al., 2001; Borrás et al., 2003; Pommel et al., 2006). It appears that the senescence signal could have been triggered by a threshold in grain filling or a decrease in leaf N (Pommel et al., 2006).

The effects of ~~conservation-agriculture~~ tillage, stubble and N management practices on the patterns of leaf senescence and the associated effects on grain yield have received little attention, especially as a pathway ~~to~~ to the improvement of NUE in maize. In wheat, ~~however, no-till~~ NT increased SPAD_{max} compared with ~~conventional-tillage~~ CT but tillage systems did not affect EC90, EC50 or the rate of senescence for individual varieties (Kitonyo et al., 2017). The timing and progression of senescence not only ~~affects~~ impacts yield but also the nutritional quality of grain (Schippers et al., 2015). Despite the weak correlations between rate of senescence and grain protein in the present study, fast rates of senescence ~~have~~ been are shown to ~~improve~~ associate with high grain protein in cereals (Xie et al., 2016). A

potentially negative consequence of delayed senescence is reduced grain protein content (Masclaux-Daubresse and Chardon, 2011).

4.3 Leaf senescence ideotype

A senescence ideotype for a short maturity maize hybrid, driven by sink size, is proposed from the present study. This ideotype ~~has had~~ high SPAD_{max} in all leaf layers, which potentially associates with higher photosynthesis (Martin et al., 2005). In the top and mid layer leaves, the onset of senescence was regulated by sink size but N fertilization delayed senescence in the bottom layer. The early onset of leaf senescence in the top and mid-layer leaves was coupled with a fast rate of senescence. ~~suggested that high~~ While high yielding hybrids should present a delay in the onset of senescence to extend photosynthetic duration, ~~coupled with~~ a fast rate of senescence ~~to hastens improve the efficiency of~~ nutrient remobilization to the grain (Wu et al., 2012). Our results contradict the delayed onset of senescence trait but high SPAD_{max} suggests the maintenance of high photosynthetic ~~rate~~ activity. Direct measurements of leaf photosynthesis are required to test this proposition.

The biochemical regulators may have strongly regulated the onset of senescence in our test hybrid (Crafts-Brandner and Poneleit, 1987). Maize derives 35-55% kernel N from post-flowering soil N uptake, while the rest is remobilised from senescing organs (Hirel et al., 2007; Gregersen et al., 2013). Increased soil N could have prolonged leaf greenness in fertilized crops compared with the unfertilized control (Rajcan and Tollenaar, 1999). Traits that promote water conservation (such as early maturity) or increase water and nutrient uptake, and high harvest index could complement the proposed senescence ideotype for a short-maturity maize hybrid (Sadras and Richards, 2014).

5. Conclusion

Understanding the factors affecting yield formation in maize is important in the management of tillage, stubble retention and N fertilization practices. In the present study, tillage and stubble retention did not affect the senescence of maize canopy. However, Understanding the patterns of leaf senescence in maize and their effects on grain yield, yield components and N remobilization efficiency can be used to guide the deployment of senescence ideotypes in conservation agriculture. N supply was the dominant factor, and interacted with stubble in driving leaf senescence. ~~The onset of senescence (EC90) did not respond to management factors but its progression (breakpoint and EC50) was regulated by growing conditions, sink size and management practices.~~ A senescence ideotype demonstrated sink-driven patterns of leaf senescence, in which faster rates of senescence associated with N supply and high yield while slower rates of senescence were concomitant with low grain yield. impacted N-use efficiency. ~~Nonetheless, these p~~Patterns of senescence ~~observed in the present study~~ could be restricted to short maturity maize hybrids. ~~Late maturity and “stay-green” hybrids are likely to exhibit different patterns. We conclude that patterns of senescence are valuable traits to explore in the improvement of grain yield and quality in conservation agriculture.~~ Further work on a wide range of senescent and “stay-green” hybrids under ~~conservation agriculture practices is required~~contrasting tillage systems and stubble amounts, and different rates and timing of N supply is required. -

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References

Abate, T., Fisher, M., Abdoulaye, T., Kassie, G.T., Lunduka, R., Marenya, P., Asnake, W., 2017.

Characteristics of maize cultivars in Africa: How modern are they and how many do
smallholder farmers grow? Agric. Food Sec. 6, 1-17.

Antonietta, M., Acciaresi, H., Guiamet, J., 2016. Responses to N deficiency in stay green and
non-stay green Argentinean hybrids of maize. J. Agron. Crop Sci. 202, 231-242.

Bänziger, M., Edmeades, G., Lafitte, H., 2002. Physiological mechanisms contributing to the
increased N stress tolerance of tropical maize selected for drought tolerance. Field
Crops Res. 75, 223-233.

Bänziger, M., Lafitte, H., 1997. Efficiency of secondary traits for improving maize for low-
nitrogen target environments. Crop Sci. 37, 1110-1117.

Barracough, P.B., Lopez-Bellido, R., Hawkesford, M.J., 2014. Genotypic variation in the
uptake, partitioning and remobilisation of nitrogen during grain-filling in wheat. Field
Crops Res. 156, 242-248.

Beyene, Y., Gowda, M., Suresh, L., Mugo, S., Olsen, M., Oikeh, S.O., Juma, C., Tarekegne,
A., Prasanna, B.M., 2017. Genetic analysis of tropical maize inbred lines for
resistance to maize lethal necrosis disease. Euphytica 213 (9), 224.

532 Biswas, A., Mandal, S., 1986. Monocarpic senescence in wheat: Influence of sterile glumes
533 and ear. *Physiol. Plant.* 67, 431-434.

534 Bonada, M., Sadras, V.O., Fuentes, S., 2013. Effect of elevated temperature on the onset and
535 rate of mesocarp cell death in berries of Shiraz and Chardonnay and its relationship
536 with berry shrivel. *Aust. J. Grape Wine Res.* 19, 87-94.

537 Bonhomme, R., 2000. Bases and limits to using 'degree. day' units. *Eur. J. Agron.* 3, 1-10.

538 Borrás, L., Maddonni, G., Otegui, M., 2003. Leaf senescence in maize hybrids: plant
539 population, row spacing and kernel set effects. *Field Crops Res.* 82, 13-26.

540 Borrel, A., Hammer, G., E, O., 2001. Stay-green: A consequence of the balance between
541 supply and demand for nitrogen during grain filling? *Ann. Appl. Biol.* 138, 91-95.

542 Borrell, A., Hammer, G., Oosterom, E., 2001. Stay-green: A consequence of the balance
543 between supply and demand for nitrogen during grain filling? *Ann. Appl. Biol.* 138,
544 91-95.

545 Brouder, S.M., Gomez-Macpherson, H., 2014. The impact of conservation agriculture on
546 smallholder agricultural yields: A scoping review of the evidence. *Agric. Ecosyst.*
547 *Environ.* 187, 11-32.

548 Capristo, P.R., Rizzalli, R.H., Andrade, F.H., 2007. Ecophysiological yield components of
549 maize hybrids with contrasting maturity. *Agron. J.* 99, 1111-1118.

550 Christopher, J.T., Veyradier, M., Borrell, A.K., Harvey, G., Fletcher, S., Chenu, K., 2014.
551 Phenotyping novel stay-green traits to capture genetic variation in senescence
552 dynamics. *Funct. Plant Biol.* 41, 1035-1048.

553 Crafts-Brandner, S.J., Poneleit, C.G., 1987. Effect of ear removal on CO₂ exchange and
554 activities of ribulose biphosphate carboxylase/oxygenase and phosphoenolpyruvate
555 carboxylase of maize hybrids and inbred lines. *Plant Physiol.* 84, 261-265.

556 Davies, P., Gan, S., 2012. Towards an integrated view of monocarpic plant senescence. Russ.
 557 J. Plant Physiol. 59, 467-478.

558 Dobermann, A., 2007. Nutrient use efficiency – measurement and management. In: Krauss,
 559 A., Isherwood, K., Heffer, P. (Eds.), Fertilizer best management practices general
 560 principles, strategy for their adoption and voluntary initiatives vs regulations.
 561 International Fertilizer Industry Association, Paris, France, pp. 1-28.

562 Escobar-Gutiérrez, A.J., Combe, L., 2012. Senescence in field-grown maize: from flowering
 563 to harvest. Field Crops Res. 134, 47-58.

564 FAO, 2015. What is conservation agriculture? <http://www.fao.org/ag/ca/1a.html>.

565 Feller, U., Anders, I., Mae, T., 2007. Rubiscolytics: fate of Rubisco after its enzymatic
 566 function in a cell is terminated. J. Exp. Bot. 59, 1615-1624.

567 French, R., Schultz, J., 1984. Water use efficiency of wheat in a Mediterranean-type
 568 environment. I. The relation between yield, water use and climate. Crop Pasture Sci.
 569 35, 743-764.

570 Gastal, F., Lemaire, G., Durand, J.-L., Louarn, G., 2015. Quantifying crop responses to
 571 nitrogen and avenues to improve nitrogen-use efficiency. In: Sadras, V.O., Calderini,
 572 D.F. (Eds), Crop physiology - Applications for genetic improvement and agronomy,
 573 2nd edn. Academic press, Elsevier, London. 161-206.

574 Gomez, K.A., Gomez, A.A., 1984. Statistical procedures for agricultural research. John
 575 Wiley & Sons, New York.

576 Gregersen, P.L., 2011. Senescence and nutrient remobilization in crop plants. In:
 577 Hawkesford, M.J., Barraclough, P. (Eds), The molecular and physiological basis of
 578 nutrient use efficiency in crops. 1st edn. Wiley Blackwell, UK. pp. 83-102.

579 Gregersen, P.L., Culetic, A., Boschian, L., Krupinska, K., 2013. Plant senescence and crop
 580 productivity. Plant Mol. Biol. 82, 603-622.

581 Harding, S.A., Guikema, J.A., Paulsen, G.M., 1990. Photosynthetic decline from high
582 temperature stress during maturation of wheat. *Plant Physiol.* 92, 648-653.

583 He, P., Osaki, M., Takebe, M., Shinano, T., Wasaki, J., 2005. Endogenous hormones and
584 expression of senescence-related genes in different senescent types of maize. *J. Exp.*
585 *Bot.* 56, 1117-1128.

586 Hirel, B., Le Gouis, J., Ney, B., Gallais, A., 2007. The challenge of improving nitrogen use
587 efficiency in crop plants: towards a more central role for genetic variability and
588 quantitative genetics within integrated approaches. *J. Exp. Bot.* 58, 2369-2387.

589 Ho, I., Below, F.E., 1989. Whole plant senescence of sunflower following seedhead removal.
590 *Plant physiol.* 91, 85-90.

591 Jaetzold, R., Schmidt, H., Hornetz, B., Shisanya, C.A., 2006. Farm management handbook of
592 Kenya. Natural conditions and farm information. Ministry of Agriculture/GTZ,
593 Nairobi, Kenya.

594 Justes, E., Mary, B., Meynard, J.-M., Machet, J.-M., Thelier-Huche, L., 1994. Determination
595 of a critical nitrogen dilution curve for winter wheat crops. *Ann. Bot.* 74, 397-407.

596 Kitonyo, O.M., Sadras, V.O., Zhou, Y., Denton, M.D., 2017. Evaluation of historic
597 Australian wheat varieties reveals increased grain yield and changes in senescence
598 patterns but limited adaptation to tillage systems. *Field Crops Res.* 206, 65-73.

599 Kitonyo, O.M., Sadras, V.O., Zhou, Y., Denton, M.D., 2018. Nitrogen fertilization modifies maize yield
600 response to tillage and stubble in a sub-humid tropical environment. *Field Crops Res.* 223,
601 113-124.

602 ~~Kitonyo, O.M., Sadras, V.O., Zhou, Y., Denton, M.D., under review. Nitrogen fertilization~~
603 ~~modifies maize yield response to tillage and stubble in a sub-humid tropical~~
604 ~~environment. Unpublished.~~

605 Lim, P.O., Kim, H.J., Gil Nam, H., 2007. Leaf senescence. *Annu. Rev. Plant Biol.* 58, 115-
606 136.

607 Liu, F., Jensen, C.R., Andersen, M.N., 2005. A review of drought adaptation in crop plants:
608 changes in vegetative and reproductive physiology induced by ABA-based chemical
609 signals. *Aust. J. Agric. Res.* 56, 1245-1252.

610 Maddonni, G., Otegui, M.E., Cirilo, A.G., 2001. Plant population density, row spacing and
611 hybrid effects on maize canopy architecture and light attenuation. *Field Crops Res.*
612 71, 183-193.

613 Martin, A., Belastegui-Macadam, X., Quilleré, I., Floriot, M., Valadier, M.H., Pommel, B.,
614 Andrieu, B., Donnison, I., Hirel, B., 2005. Nitrogen management and senescence in
615 two maize hybrids differing in the persistence of leaf greenness: agronomic,
616 physiological and molecular aspects. *New Phytol.* 167, 483-492.

617 Masclaux-Daubresse, C., Chardon, F., 2011. Exploring nitrogen remobilization for seed
618 filling using natural variation in *Arabidopsis thaliana*. *J. Exp. Bot.* 62, 2131-2142.

619 Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L.,
620 Suzuki, A., 2010. Nitrogen uptake, assimilation and remobilization in plants:
621 challenges for sustainable and productive agriculture. *Ann. Bot.* 105, 1141-1157.

622 Masclaux-Daubresse, C., Reisdorf-Cren, M., Orsel, M., 2008. Leaf nitrogen remobilisation
623 for plant development and grain filling. *Plant Biol.* 10, 23-36.

624 Monsanto, 2017. DK8031, the hybrid for food security. <http://www.monsantoafrica.com>

625 Noodén, L.D., Guiamét, J.J., John, I., 1997. Senescence mechanisms. *Physiol. Plant.* 101,
626 746-753.

627 Plénet, D., Lemaire, G., 1999. Relationships between dynamics of nitrogen uptake and dry
628 matter accumulation in maize crops. Determination of critical N concentration. *Plant*
629 *Soil* 216, 65-82.

630 Pommel, B., Gallais, A., Coque, M., Quillere, I., Hirel, B., Prioul, J., Andrieu, B., Floriot, M.,
 631 2006. Carbon and nitrogen allocation and grain filling in three maize hybrids differing
 632 in leaf senescence. *Eur. J. Agron.* 24, 203-211.

633 Rajcan, I., Tollenaar, M., 1999. Source: sink ratio and leaf senescence in maize: I. Dry matter
 634 accumulation and partitioning during grain filling. *Field Crops Res.* 60, 245-253.

635 Sadras, V., Echarte, L., Andrade, F., 2000. Profiles of leaf senescence during reproductive
 636 growth of sunflower and maize. *Ann. Bot.* 85, 187-195.

637 Sadras, V.O., Richards, R.A., 2014. Improvement of crop yield in dry environments:
 638 benchmarks, levels of organisation and the role of nitrogen. *J. Exp. Bot.* 65, 1981-
 639 1995.

640 Schippers, J.H., Jing, H.-C., Hille, J., Dijkwel, P.P., 2007. Developmental and hormonal
 641 control of leaf senescence. In: Gan, S. (Ed), *Senescence processes in plants*. 1st Edn.
 642 Blackwell publishing, New York, USA. pp. 145-170.

643 Schippers, J.H., Schmidt, R., Wagstaff, C., Jing, H.-C., 2015. Living to die and dying to live:
 644 the survival strategy behind leaf senescence. *Plant physiol.* 169, 914-930.

645 Sinclair, T., Bennett, J., Muchow, R., 1990. Relative sensitivity of grain yield and biomass
 646 accumulation to drought in field-grown maize. *Crop Sci.* 30, 690-693.

647 Staswick, P.E., 1992. Jasmonate, genes, and fragrant signals. *Plant Physiol.* 99, 804-807.

648 Thomas, H., Ougham, H., 2015. Senescence and crop performance. In: Sadras, V.O.,
 649 Calderini, D.F. (Eds.), *Crop physiology: Applications for genetic improvement and*
 650 *agronomy*. Elsevier, London, pp. 223-249.

651 Valentinuz, O.R., Tollenaar, M., 2004. Vertical profile of leaf senescence during the grain-
 652 filling period in older and newer maize hybrids. *Crop Sci.* 44, 827-834.

653 van Doorn, W.G., Woltering, E.J., 2004. Senescence and programmed cell death: substance
 654 or semantics? *J. Exp. Bot.* 55, 2147-2153.

- Verhulst, N., Govaerts, B., Nelissen, V., Sayre, K.D., Crossa, J., Raes, D., Deckers, J., 2011. The effect of tillage, crop rotation and residue management on maize and wheat growth and development evaluated with an optical sensor. *Field Crops Res.* 120, 58-67.
- Wei, H., Meng, T., Li, X., Dai, Q., Zhang, H., Yin, X., 2018. Sink-source relationship during rice grain filling is associated with grain nitrogen concentration. *Field Crops Res.* 215, 23-38.
- Wilkinson, S., Davies, W.J., 2002. ABA-based chemical signalling: the co-ordination of responses to stress in plants. *Plant Cell Environ.* 25, 195-210.
- Wolfe, D., Henderson, D., Hsiao, T., Alvino, A., 1988. Interactive water and nitrogen effects on senescence of maize. I. Leaf area duration, nitrogen distribution, and yield. *Agron. J.* 80, 859-864.
- Wu, X.Y., Kuai, B.K., Jia, J.Z., Jing, H.C., 2012. Regulation of Leaf Senescence and Crop Genetic Improvement. *J. Integr. Plant Biol.* 54, 936-952.
- Xie, Q., Mayes, S., Sparkes, D.L., 2016. Early anthesis and delayed but fast leaf senescence contribute to individual grain dry matter and water accumulation in wheat. *Field Crops Res.* 187, 24-34.
- Yang, J., Udvardi, M., 2017. Senescence and nitrogen use efficiency in perennial grasses for forage and biofuel production. *J. Exp. Bot.* [69, 855-865](#). [doi:10.1093/jxb/erx241](#)