

Estimate App for Yam Anthracnose Disease Phenotyping: User Guide

Olufisayo Kolade and P. Lava Kumar



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and Bananas**

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Cover: Yam anthracnose phenotyping using Estimate app.
Inset. Symptoms of yam anthracnose disease.



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Preface

Yam anthracnose disease (YAD), caused by the *Colletotrichum gloeosporioides*, is an important disease of yam (*Dioscorea* spp.). The water yam, *Dioscorea alata*, is known to be more susceptible to this soil-borne pathogen of ubiquitous occurrence. Severe YAD at the early stage of crop growth can cause up to 70% tuber yield loss. The use of resistant cultivars is the most cost-effective method for preventing economic losses due to YAD. Breeding programs screen yam genotypes for YAD resistance under natural field conditions or artificial sick plots. Genotype screening can also be performed under screen house conditions or artificial inoculation on the whole plant or detached leaves under laboratory conditions.

The response of genotypes to YAD is assessed by measuring symptom severity using a 1 to 5 severity rating scale. Conventional scoring for the severity of the infection are liable to errors and bias because they depend on the rater's experience. We developed the "Estimate" mobile app to improve the accuracy of data collection and reduce rater bias. Estimate app enhances the accuracy of YAD severity assessment and digitalizes data for further analysis.

This manual provides step-by-step instructions for using the Estimate app to assess YAD severity on whole plants or detached leaf assays. It is a valuable tool for technicians and researchers working on yam for an accurate and reliable rating of infection, and it also simplifies data collection, storage, and sharing. This user guide provides step-by-step details of the mode of operation of the App.

Acknowledgments

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Introduction

Yam anthracnose disease caused by *Colletotrichum gloeosporioides* is an economically important disease capable of lowering tuber yields and sometimes cause premature death of the plant. YAD symptoms include dark brown necrotic lesions with yellow margins (Fig 1d). The necrotic areas coalesce to form blight in susceptible genotypes (Fig 1). Breeding programs are ongoing to develop yam varieties with durable YAD resistance. Yam plants are evaluated for YAD response under field, screen house conditions, or laboratory using the Detached Leaf Assay (DLA) (Kolade et al., 2018). DLA method is fast (it takes 3 weeks to complete) and offers a high-throughput evaluation of breeding population or landraces under laboratory conditions.

Symptom-based phenotyping is an important procedure for assessing the yam plant response to YAD under natural field conditions or artificial inoculation in the field or screen house or using DLA as a proxy to the whole plant assay under laboratory conditions. The assessment of plant response to fungal infection is based on the symptom severity (percent leaf or plant area damaged relative to the total area) assessed using a symptom severity rater scale of 1 to 5 at regular time intervals. As per the conventional rating scale, plants (or leaves in case of DLA) showing no symptoms are rated as score 1; plants with necrotic spots or blight symptoms covering on 1 to 25% of the leaves, 26 to 50%, 51 to 75% and >75% as score 2, score 3, score 4, and score 5, respectively. The symptom severity data is then used for the categorization of genotypes as resistant (R) or susceptible (S) based on a scale of 1 to 5, where 1 = immune, 2 = resistant, 3 = moderately resistant, 4 = susceptible, and 5 = highly susceptible (Onyeka et al., 2006; Aduramigba-Modupe et al., 2008; Nwadili et al., 2017).

Visual scoring of plant disease severity is generally dependent on the experience of the rater and liable to rater bias and person-to-person variation. Accuracy of phenotyping is crucial for genotype

categorization, including studies on the genetics of resistance, marker development, and other relevant studies.

To address this challenge, improve efficiency, and reduce rater bias during YAD phenotyping, an ICT-based application, the Estimate, was adopted (Pethybridge and Nelson, 2017). The App uses reference images of known severities to improve the accuracy and precision of disease severity estimation by visually relating them to what is seen on symptomatic plants.

“Estimate”: An image analysis App

The Estimate is an easy-to-use smartphone application (usable on iMac and Android operating systems) to assist with the YAD disease severity assessment using a repository of standard area diagrams designed for specific plant diseases (Pethybridge and Nelson, 2017; Kolade and Kumar, 2019). The App provides a range of images of defined diseased severities enabling comparisons between estimated disease severity on a yam leaf (sampling unit) and selecting the most representative in the standard area diagram (SAD). Each image represents a particular severity rating scale or a range for percent damage area. Users score by comparing and matching diseased leaves on the field, screen house, or laboratory with SADs on the App to estimate the disease severity easily.

The Estimate app improves the accuracy of data collection and reduces rater bias (Bock et al., 2008; 2009). It also offers additional advantages of digital data collection and data sharing in CSV format, which can be converted to XLS format for further analysis. The disease severity assessment can be made by referring to a linear or logarithmic scale and data recorded as ordinal or continuous. The App allows users to save data on the device or share spreadsheets in the CSV format by e-mail. Users can enter data as single samples or in groups according to the layout of the field experiment.

The Estimate app includes a semi-automatic program that guides users on using the App for phenotyping YAD symptom severity in the field or DLA application in the laboratory.

Two versions of SADs are available on the Estimate app for yam anthracnose disease to support (i) evaluation in the laboratory using DLA (Fig. 2) and (ii) evaluation under field (Fig. 3). This handbook details how to use the Estimate App for the assessment of the yam anthracnose disease.

- **Start Screen:** Provides instructions to set the App for YAD scoring.
- **Begin Assessment Screen:** Provides instructions to select the standard area diagrams (reference images) of the select pathosystem
- **Sampling Unit Screen:** This screen enables the user to collect the required metadata associated with the assessment:
 - 1 Date (pre-populated)
 - 2 Time (pre-populated)
 - 3 Location of disease assessment (can be pre-populated via geospatial position, when the device is activated to collect location)
 - 4 Experimental treatments (if applicable)
 - 5 Number of plots, subplots, and sampling units

Information on the disease and pathogen is pre-populated following the specific standard area diagram selection on the previous screen.

Estimate Disease Screen. This provides instructions and examples of disease severities for the selected pathosystems. The user compares the disease severity on a specific sampling unit to the standard area diagram (reference image) and selects the closest match. Once the assessments have been completed, the data can be saved as a CSV file within the device and shared using e-mail or other file-sharing applications.

This manual provides step-by-step details to use Estimate app for YAD phenotyping.



a



b



c



d

Figure 1. Healthy and infected *Dioscorea alata* plants in the field (a and b) and in the laboratory (DLA) (c and d).

ESTIMATE DISEASE

Scale Logarithmic Ordinal | Plot 1/20 | Plant 1/100 | Leaf 1/15



0% disease



1 - 2% disease



3 - 5% disease



6 - 11% disease



12 - 24% disease



25 - 49% disease



50 - 74% disease



75 - 87% disease



88 - 93% disease



94 - 96% disease



97 - 99% disease



100%

disease

100% disease



Back



Missing



End

Figure 2. Standard area diagrams for YAD assessment in the laboratory.

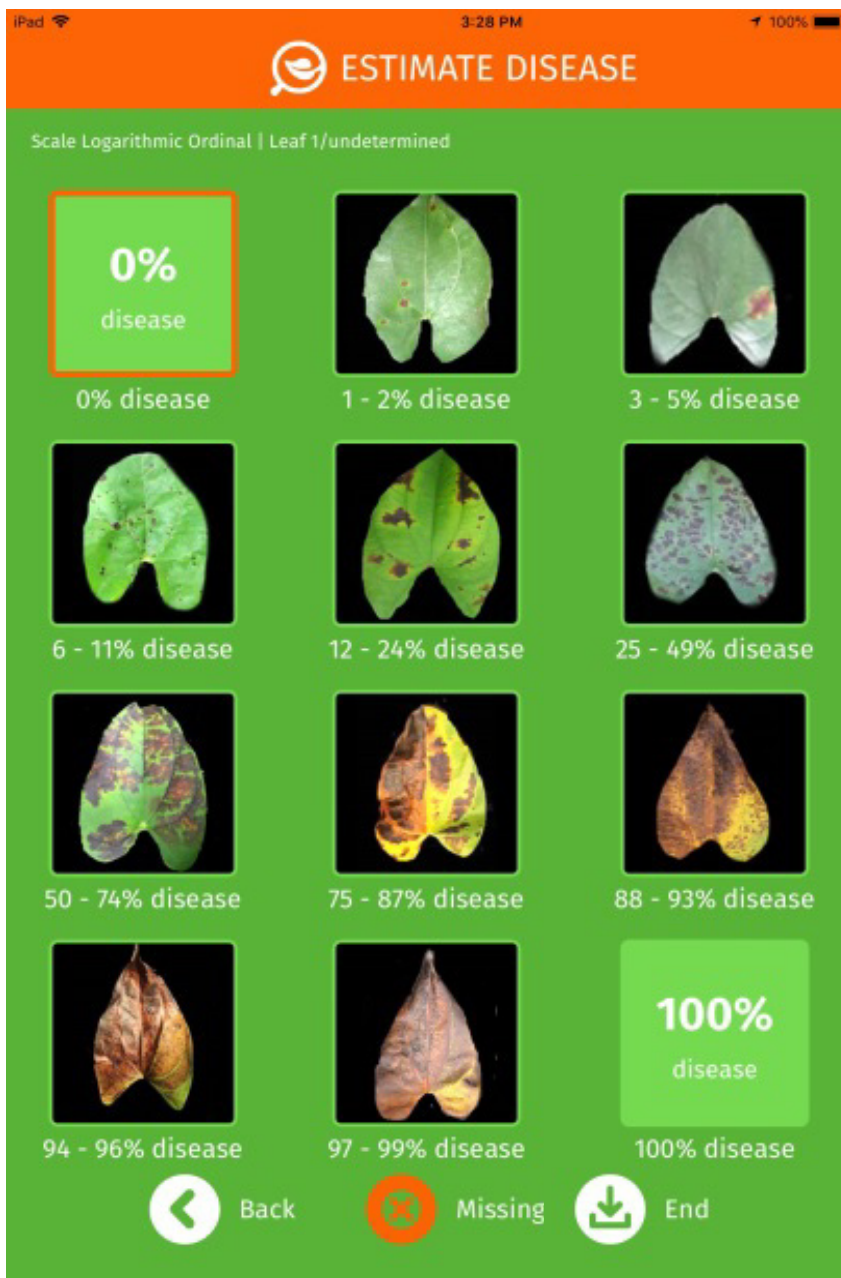


Figure 3. Standard area diagrams for YAD assessment on the field.

Step-by-step details for using the Estimate app

- 1 Download the App from the Apple play store <https://itunes.apple.com/in/app/estimate-disease-severity-assessment/id1193605571?mt=8> or the Google play store for the android version (Note that the android version is not activated yet, but a link can be obtained from authors).

The workflow for the operation of the App is shown in Figure 4.

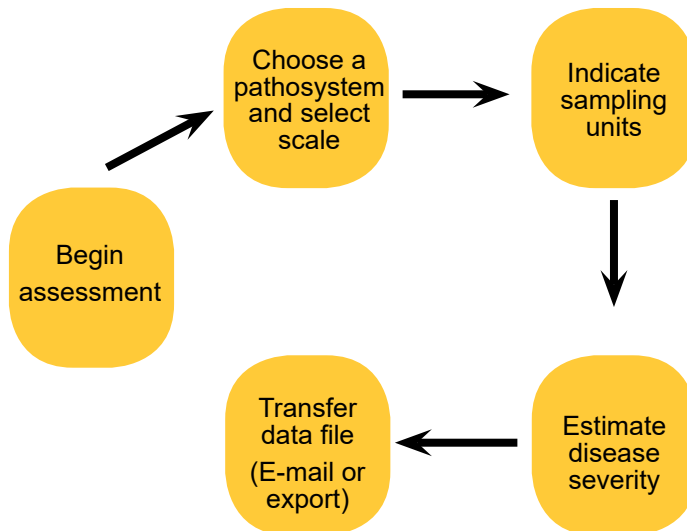


Figure 4. Workflow of the use of the Estimate app for YAD screening.

2 Follow the instructions as shown in Figures 5–18.

Selection of Estimate app



Figure 5. Home screen of iPad.

Starting assessment

iPad 4:18 PM 24%

ESTIMATE

	15 Nov 2018	8:47:46 AM	Yam anthracnose	Logarithmic Ordinal
	15 Nov 2018	10:38:56 AM	Yam anthracnose	Logarithmic Ordinal
	15 Nov 2018	11:01:21 AM	Yam anthracnose	Logarithmic Ordinal
	16 Nov 2018	9:04:13 AM	Yam anthracnose	Logarithmic Ordinal
	19 Nov 2018	3:16:32 PM	Yam anthracnose	Logarithmic Ordinal
	19 Nov 2018	3:55:41 PM	Yam anthracnose	Logarithmic Ordinal
	21 Nov 2018	9:11:43 AM	Yam anthracnose	Logarithmic Ordinal
	23 Nov 2018	8:51:33 AM	Yam anthracnose	Logarithmic Ordinal
	26 Nov 2018	7:54:49 AM	Yam anthracnose	Logarithmic Ordinal
	26 Nov 2018	10:01:32 AM	Yam anthracnose	Logarithmic Ordinal
	27 Nov 2018	8:49:53 AM	Yam anthracnose	Logarithmic Ordinal
	27 Nov 2018	1:58:27 PM	Yam anthracnose	Logarithmic Ordinal

About
Click to obtain more information about App

Instructions
Click to obtain instructions on how to use App

Begin Assessment
Click to begin assessment

Figure 6. Welcome page with data list and navigating details.

Choice of pathosystem and scale of measurement

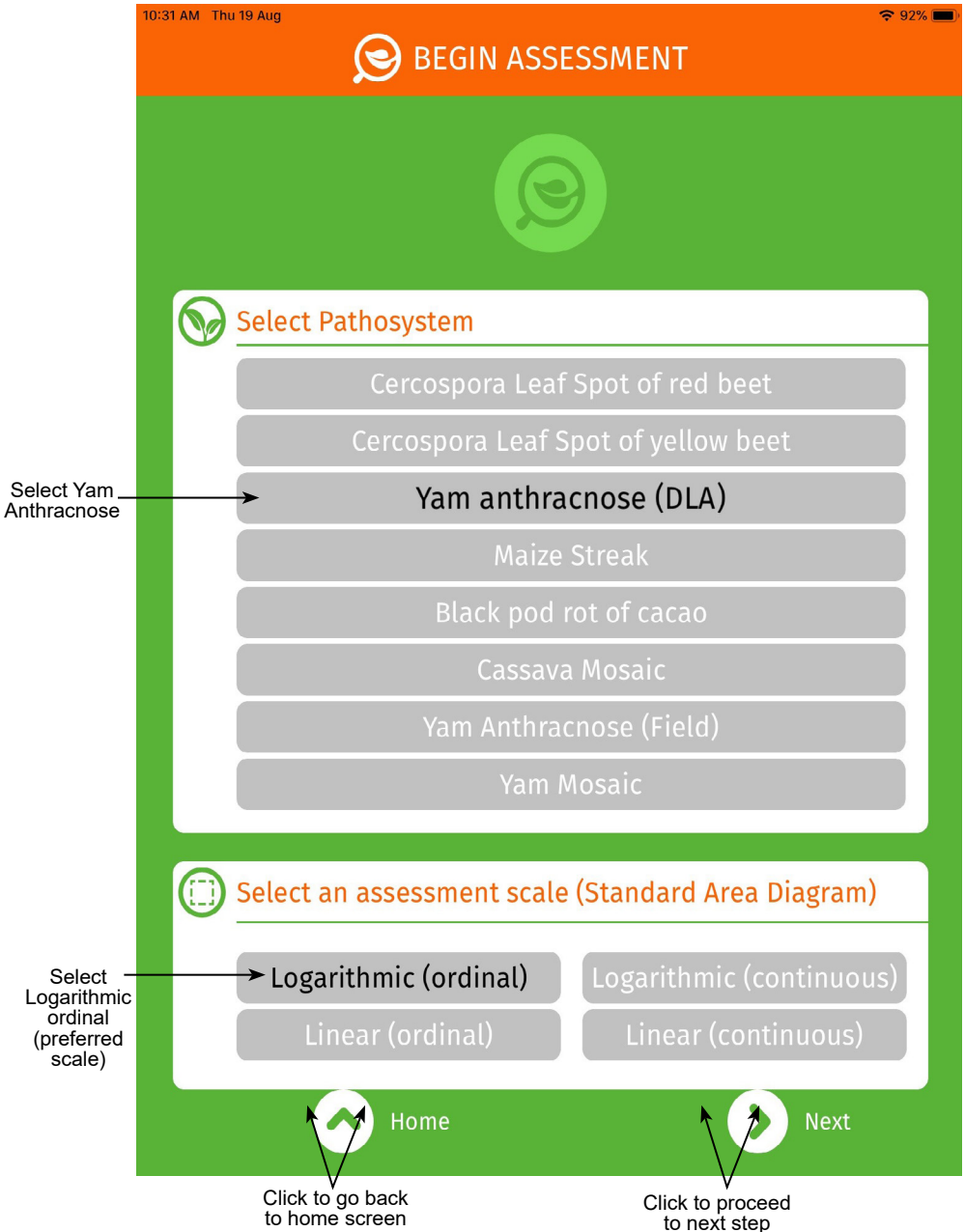


Figure 7. Begin assessment page.

Filling in sample units

11:28 Tue 27 Jul 48%

SAMPLING UNITS

Date: 27 Jul 2021

Time: 11:25:18

Location: (7.498810126158607,3.906351014405318)

Treatment: none

Disease name: Yam anthracnose (DLA)

Pathogen name: Colletotrichum gloeosporioides

Host name: Yam (Dioscorea spp)

Experiment ID: TDa92-2

Other information: Breeding mother block assessment

Number of plots: 20

Sub-plots: 0

Number of plants: 100

Number of leaves: 15

Back Estimate Disease

Supply relevant information (some details are auto populated)

Click on back to go to home page

Select "Estimate Disease" to go to Standard Area Diagrams' page

Figure 8a. Sampling unit's setting page.

Date	Auto field. App extract this information from your mobile device	→ Date:	10 M
Time	Auto field. App extract this information from your mobile device	→ Time:	4:18:
Location	Auto field. App extract GPS information from your mobile device (make sure to turn on "location". Otherwise, GPS information do not appear	→ Location:	(7.49
Treatment	Indicate treatment information if applicable, otherwise leave it blank	→ Treatment:	none
Disease name	Auto field	→ Disease name:	Yam
Pathogen name	Auto field	→ Pathogen name:	Yam
Host name	Auto field	→ Host name:	Yam
Variety name	Specify	→ Variety name:	TDa9
Other information	Specify any details important for this assessment. Keep it short (8 words or less)	→ Other information:	Breed
Number of plots	Specify (if applicable, otherwise leave it zero; you can type or slide the bar)	→ Number of plots:	20
Sub-plots	Specify (if applicable, otherwise leave it zero; you can type or slide the bar)	→ Sub-plots:	0
Number of plants	Specify (indicate number of plants to be scored; you can type or slide the bar)	→ Number of plants:	100
Number of leaves	Specify (indicate number of leaves to be scored; you can type or slide the bar)	→ Number of leaves:	15
			Back

Figure 8b. Further details of sampling unit's setting page.

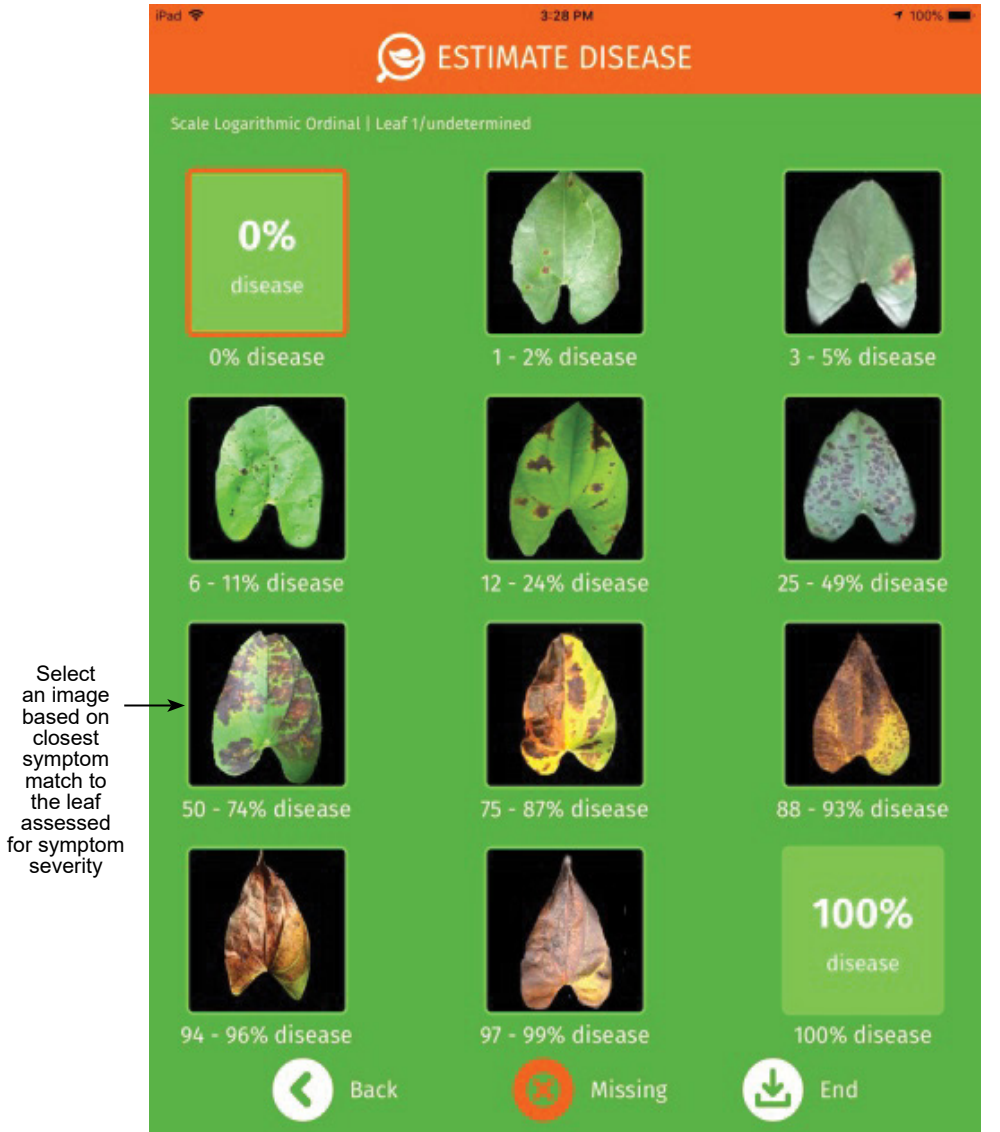
Estimating disease severity for laboratory assays



Click on appropriate icons to navigate back, indicate missing data, and end the data collection

Figure 9. Standard area diagrams for detached leaf assay (DLA).

Estimating disease severity for field assays



Click on appropriate icons to navigate back, indicate missing data, and end the data collection

Figure 10. Standard area diagrams for field-based evaluations of YAD severity.

Viewing / editing data

The screenshot shows the ASSESSMENT app interface on an iPad. At the top, there is a status bar with 'iPad', signal strength, '4:31 PM', and '22%' battery. Below the status bar is an orange header with the ASSESSMENT logo. The main content area is green and contains several data entry fields: Date (10 Mar 2021), Time (4:29:30 PM), Location (7.49837219317167,...), Disease (Yam anthracnose), Host (Yam), and Variety (TDa 92-2). Below these fields is a table with columns: Treatment, Plot, Subplot, Plant, Leaf, and Severity (%). The table contains 11 rows of data. At the bottom of the table is an 'End Process' button with a play icon. Two arrows point from the caption below to the 'End Process' button and the 'Severity (%)' column.

Treatment	Plot	Subplot	Plant	Leaf	Severity (%)
none			1	1	18.50%
			1	2	81.50%
			1	3	62.50%
			1	4	91.00%
			1	5	37.50%
			1	6	4.50%
			1	7	18.50%
			1	8	9.00%
			1	9	0.00%
			1	10	1.50%
			1	11	18.50%

Click to go back to file list page

Click to edit submitted data (it will take you to SAD page)

Figure 11. Data view page (scores for leaves 1–11).

Viewing / editing data cont'd

iPad 4:31 PM 22%

ASSESSMENT

Date: 10 Mar 2021 Time: 4:29:30 PM Location: (7.49837219317167,...

Disease: Yam anthracnose Host: Yam Variety: TDa 92-2

Treatment	Plot	Subplot	Plant	Leaf	Severity (%)
			1	5	37.50%
			1	6	4.50%
			1	7	18.50%
			1	8	9.00%
			1	9	0.00%
			1	10	1.50%
			1	11	18.50%
			1	12	0.00%
			1	13	81.50%
			1	14	0.00%
			1	15	81.50%

End Process

Click to go back to file list page

Click to edit submitted data (it will take you to SAD page)

Figure 12. Data view page (scores for leaves 5–15).

Sending / exporting a data file

The screenshot shows the ESTIMATE app interface. At the top, the status bar displays '14:16 Wed 8 Sep' and '49%' battery. The app header is orange with the ESTIMATE logo. The main content is a green list of data entries. Each entry includes a leaf icon, a date and time, the species name 'Colletotrichum gloeosporioides', and the model 'Logarithmic Ordinal'. The last entry is highlighted with a blue 'Email' button and an orange 'Export' button. An arrow points from the text 'Swipe right and click to send file by e-mail' to the 'Email' button. At the bottom, there are three circular icons: a question mark for 'About', an information icon for 'Instructions', and the ESTIMATE logo for 'Begin Assessment'.

Date	Time	Species	Model
19 Aug 2021	10:31:15	Colletotrichum gloeosporioides	Logarithmic Ordinal
26 Aug 2021	10:31:05	Colletotrichum gloeosporioides	Logarithmic Ordinal
26 Aug 2021	10:58:32	Colletotrichum gloeosporioides	Logarithmic Ordinal
26 Aug 2021	11:31:41	Colletotrichum gloeosporioides	Logarithmic Ordinal
31 Aug 2021	09:37:14	Colletotrichum gloeosporioides	Logarithmic Ordinal
31 Aug 2021	09:44:45	Colletotrichum gloeosporioides	Logarithmic Ordinal
31 Aug 2021	10:34:01	Colletotrichum gloeosporioides	Logarithmic Ordinal
3 Sep 2021	16:02:42	Colletotrichum gloeosporioides	Logarithmic Ordinal
6 Sep 2021	09:51:53	Colletotrichum gloeosporioides	Logarithmic Ordinal
8 Sep 2021	08:56:03	Colletotrichum gloeosporioides	Logarithmic Ordinal
8 Sep 2021	10:02:35	Colletotrichum gloeosporioides	Logarithmic Ordinal
8 Sep 2021	10:08:06	Colletotrichum gloeosporioides	Logarithmic Ordinal

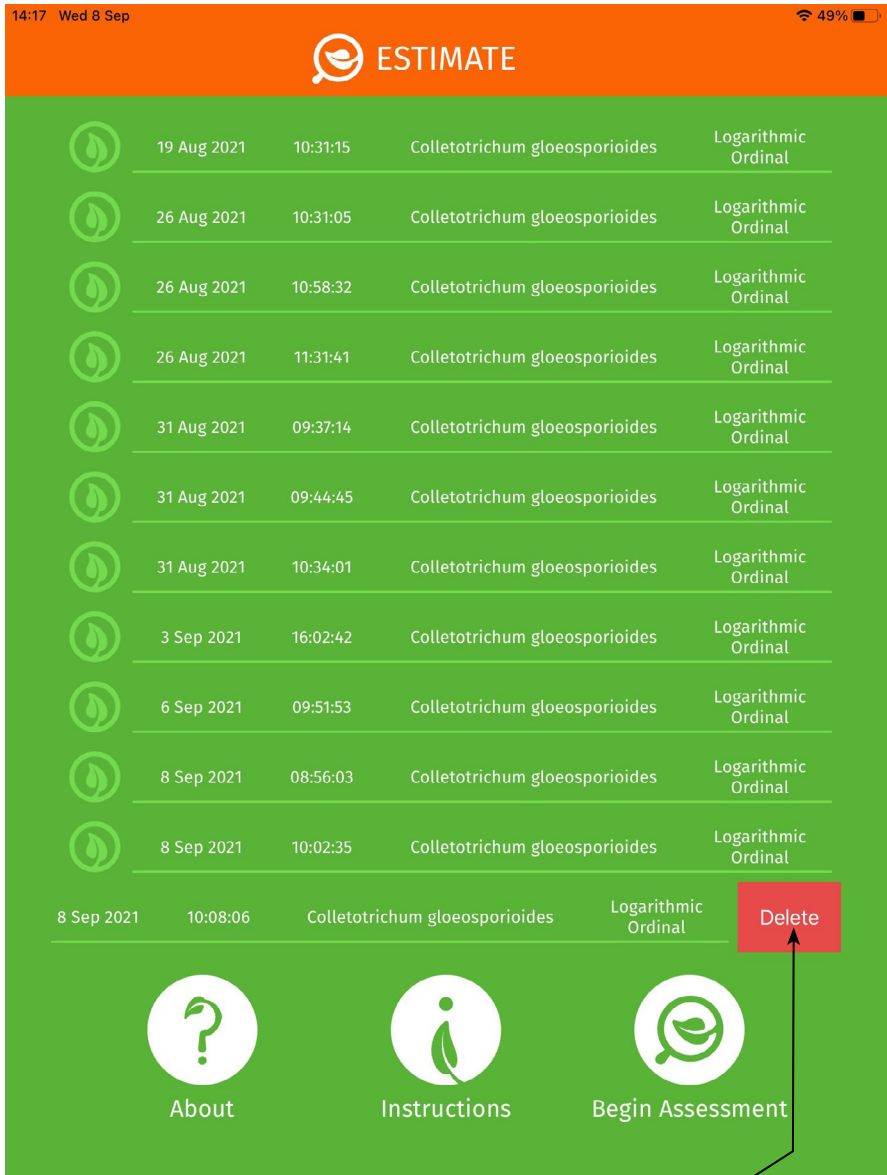
Swipe right and click to send file by e-mail

Email Export

About Instructions Begin Assessment

Figure 13. Data list page indicating how to transfer a file.

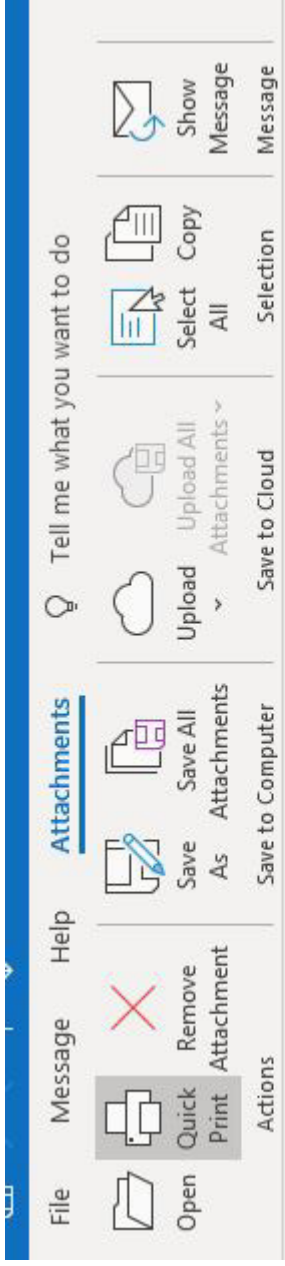
Deletion of a data file



Swipe left and click to delete unwanted file

Figure 14. Data field deletion option.

Data file sharing from Estimate app as an e-mail attachment



Assessment details



Swipe right and
click to send
file by e-mail

Figure 15. E-mail view of data.

Viewing data output on Excel or CSV spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1											
2		Date: 10 Mar 2021	Time: 4:29:30 PM	Location: (7.49837219317167,3.90697694073067)							
3		Disease: Yam anthracnose	Host: Yam	Variety name: TDa 92-2	Standard area diagram style: Logarithmic Ordinal						
4		Treatment: none	Plot	Plant	Leaf	Severity (%)					
5					1	1	18.50%				
6					1	2	81.50%				
7					1	3	62.50%				
8					1	4	91.00%				
9					1	5	37.50%				
10					1	6	4.50%				
11					1	7	18.50%				
12					1	8	9.00%				
13					1	9	0.00%				
14					1	10	1.50%				
15					1	11	18.50%				
16					1	12	0.00%				
17					1	13	81.50%				
18					1	14	0.00%				
19					1	15	81.50%				

Displays file information

Figure 16. YAD severity data view in MS Excel.

3 Laboratory assessment

Score at least 3 replicates of yam leaves directly or score pictures of detached leaf assay using the photographic standard area diagrams (SADs) (shown in Fig. 2). Figure 17 shows the typical leaves symptoms obtainable from a detached leaf assay with respective scores from the Estimate app.

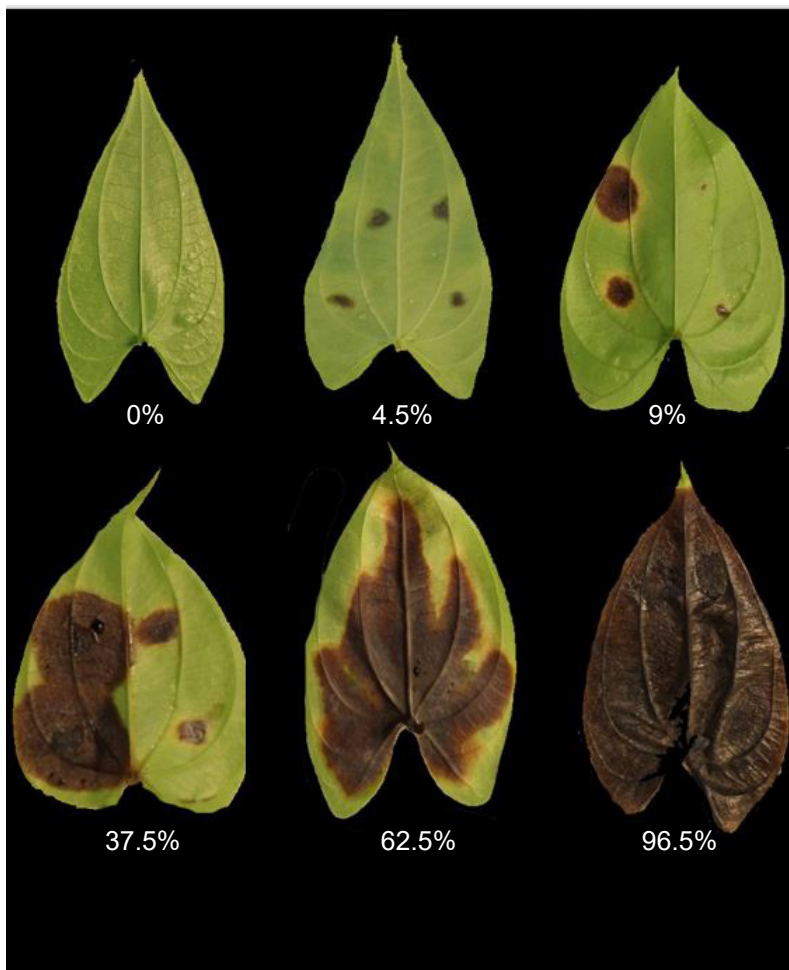
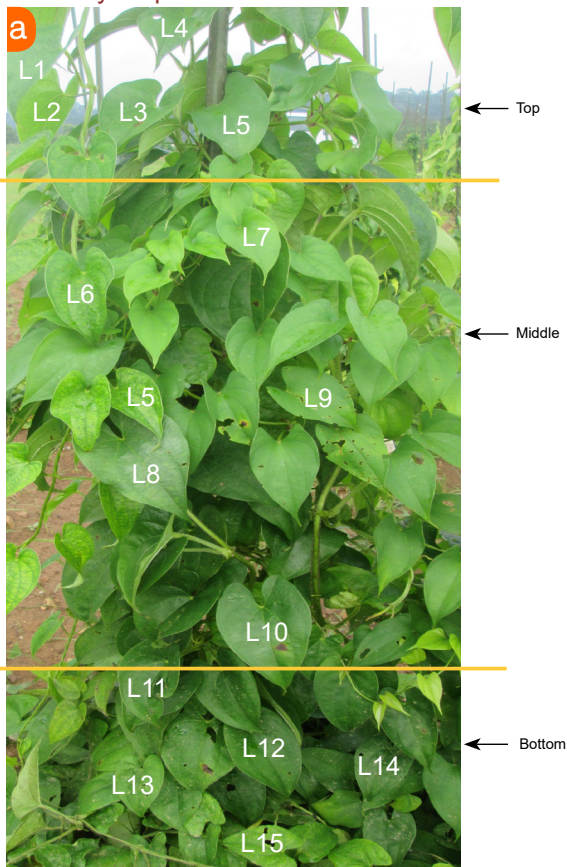


Figure 17. YAD symptoms of DLA of yam and their mid-point values based on the Estimate app.

4 Field assessment
 Users are advised to follow a standard pattern for scoring leaf sections on the plant. At IITA, 15 leaves per plant consisting of 5 leaves (with most common YAD symptoms) each from the top, middle and bottom portions of the plant. Figure 18a and 18b show the typical leaves assessed for both staked and unstaked plants. Calculate the mean severity, to have an overall assessment of the plant using the SADs for the field. Figure 19 shows typical YAD severity scores obtainable by visual rating.

Staked yam plant



Unstaked yam plant

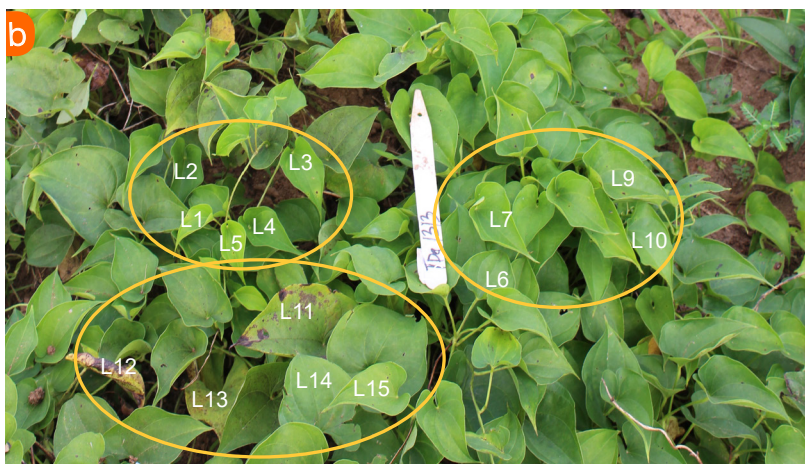


Figure 18. Pattern for scoring leaves of staked plants (a) and non-staked plants (b).

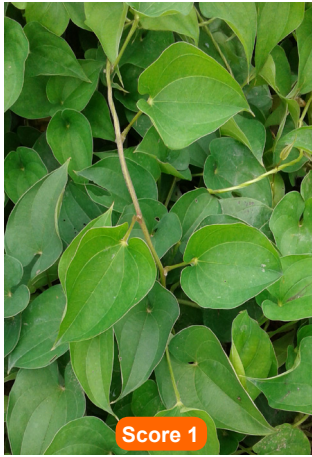


Figure 19. YAD-affected *Dioscorea alata* plants rated based on the symptom severity.

5 Correction of wrong entry and missing data

Select the entry to correct, choose the right SAD, then end process, (“Back” may wipe off previous data, especially in the Android version). For missing plant, or when leaves are not up to 15, click severally on the “missing” button to indicate the number of leaves or plants missing from a particular plot based on the user’s discretion.

6 Data collation and data formatting

Data is usually obtained in a comma separated value (CSV) format. Data are obtained as mid-point values of the ranges shown on the “estimate disease” interface of the App (Table1).

The percentage data obtained can be transformed to values representing a scale of 1 to 5, where 1 = no symptoms or 0%, 2 = 0.1–25%, 3 = 25.1–50%, 4 = 50.1–75%, and 5 = >75% (75.1- 100%) (Table 2) or can be used directly as percentage values.

Table 1. The midpoint values for the logarithmic ordinal scale.

Estimate value range (%)	Midpoint values (%)
0	0
1–2	1.5
3–5	4.5
6–11	9
12–24	18
25–49	37.5
50–74	62.5
75–87	81.5
88–93	91.0
94–96	96.5
97–99	98.5
100	100

7 Working with CSV data file generated by Estimate app and data analysis:

1. Download CSV file generated by Estimate app and save it as a Microsoft Excel file. If the data obtained from the App have “%” suffix (see Figure 20A), convert data to remove “%” (as illustrated in Figure 20B) using the formula, $=\text{LEFT}(X1,5) * 100$, where LEFT is an MS Excel function for specifying the number of characters in a cell, X1 represents the value in the cell to be converted (for example C2 in Figure 20), and 5 is the number of characters needed.
2. Write out the formula (example in G1) in “I1” with the first X1 value and paste as formula for all the remaining cells for data conversion. All the data will be converted to plain text (without %) (Figure 20).

Skip this conversion step for data without % symbol.

A. Raw data								B. Converted data		
A	B	C	D	E	F	G	H	I	J	K
SN	Accession ID	YAD severity @Day3 (X1)	YAD severity @Day9 (X2)	YAD severity @Day15 (X3)				YAD severity @Day3 (X1)	YAD severity @Day9 (X2)	YAD severity @Day15 (X3)
1	Accn1	4.5%	10%	18.50%		$=\text{LEFT}(C2,5) * 100$		4.5	10	18.5
2	Accn2	0%	4.5%	9%				0	4.5	9
3	Accn3	4.5%	62.50%	100%				4.5	62.5	100
4	Accn4	3%	4.50%	9%				3	4.5	9
5	Accn5	1.50%	3.50%	4.50%				1.5	3.5	4.5
6	Accn6	3.50%	37.50%	62.50%				3.5	37.5	62.5
7	Accn7	9%	9%	37.50%				9	9	37.5
8	Accn8	0%	1.50%	4.50%				0	1.5	4.5
9	Accn9	1.50%	1.50%	1.50%				1.5	1.5	1.5
10	Accn10	18.50%	25%	100%				18.5	25	100
11	R	0%	0%	0%				0	0	0
12	S	100%	100%	100%				100	100	100

Figure 20. Example data set to demonstrate conversion of values with % (A) into normal values (B) using the formula detailed in Section 7. (Acc1 to 10 are accession numbers of the test genotypes; R=Resistant Check and S=Susceptible Check. Data in columns C, D and E are raw values and values in I, J and K are converted values).

3. Analyze data as per the pre-established method of your choice.
4. In Section 8, methods used for the classification of yam accessions based on YAD severity scores as resistant or susceptible are described.

8 Working with YAD severity data generated by Estimate app/data analysis contd'.

1. Estimation of mean incidence and mean severity

- (a) For field data, if the assessment was done as Top (T), Middle (M) and Bottom (B), estimate mean severities for each portion as well as whole plant mean by taking the mean of the T, M, B values.
- (b) For DLA, estimate means of the replicates of an accession
As an option, the mean severity data can be converted into 1 to 5 rating scale, and the same can be used for categorizing accessions (Table 2).

Table 2. Rating scale of YAD severity.

Estimate severity range (%)	Equivalent conventional rating scale	Status Description
0	1	Immune
0.1 to 25	2	Resistant
25.1 to 50	3	Moderately Resistant
50.1 to 75	4	Susceptible
75.1 to 100	5	Highly Susceptible

2. Estimation of area under the disease progression curve (AUDPC):

It is recommended to estimate AUDPC scores for accessions classification.

Use mean severity scores of each observation to estimate AUDPC values as per the method described below:

$$AUDPC = \sum \left[\frac{(X_1 + X_2)}{2} \right] x (t_2 - t_1)$$

Where X_1 is the disease severity at time 1 (t_1), X_2 is the disease severity at time 2 (t_2) and $(t_2 - t_1)$ = time interval between the two readings.

To calculate AUDPC using MS Excel:

- i. Create an MS Excel template for data analysis. This is further explained using an example data set of DLA-based assessment of 10 yam accessions (see Figure 21). The data set includes percent YAD severity scores at observation 1 at day 3 (column B), observation 2 at day 9 (column C), and observation 3 at day 15 (column D) for 10 accessions (rows 2 to 11), a resistant check (R-check; row 12) and a susceptible check (S-check; row 13).
- ii. Calculate the mean of the first two YAD severity scores as shown in Column F, and the time interval between the two observations as shown in Column G. Multiply the two values to obtain AUDPC1 as shown in Column H. Repeat the same process to estimate AUDPC2 values (Column K). Add AUDPC1 and AUDPC2 to get the total AUDPC score (Column L). The AUDPC scores can be used for the classification of genotypes.

Interpretation based on AUDPC values:

A low AUDPC score is an indication that a genotype is resistant or the disease progressed slowly (less susceptible). In contrast, a high AUDPC score indicates rapid progression of the disease and implies high susceptibility. Use known resistant and susceptible checks to set thresholds for selection.

3. Estimation of rAUDPC

Relative AUDPC (rAUDPC) is preferred, especially to compare data generated from multiple seasons or experiments performed at different time intervals. The rAUDPC involves the use of the “Maximum Potential AUDPC (MPA),” which is the AUDPC of a genotype can have if it has a 100% severity score (see row 13); that is the worst-case scenario.

	A	B	C	D	E	F	G	H	I	J	K	L	M
	Genotype I.D	YAD O1 @D3 X1	YAD O2 @D9 X2	YAD O3 @D15 X3		$(X1+X2)/2$	$t2-t1 = \text{day}(@D9 - @D3)$	AUDPC1 $(F2 * G2)$	$(X2+X3)/2$	$t3-t2 = \text{day}(@D15 - @D9)$	AUDPC2 $(I2 * J2)$	AUDPC $(H2+K2)$	rAUDPC $(L2/(G2*100 + J2*100))$
1													
2	Acc1	5.0	10.0	20.0		7.5	6.0	45.0	15	6.0	90.0	135.0	0.1
3	Acc2	0.0	5.0	10.0		2.5	6.0	15.0	7.5	6.0	45.0	60.0	0.1
4	Acc3	5.0	40.0	60.0		22.5	6.0	135.0	50	6.0	300.0	435.0	0.4
5	Acc4	10.0	30.0	40.0		20	6.0	120.0	35	6.0	210.0	330.0	0.3
6	Acc5	15.0	50.0	60.0		32.5	6.0	195.0	55	6.0	330.0	525.0	0.4
7	Acc6	0.0	25.0	65.0		12.5	6.0	75.0	45	6.0	270.0	345.0	0.3
8	Acc7	25.0	40.0	40		32.5	6.0	195.0	40	6.0	240.0	435.0	0.4
9	Acc8	0.0	5.0	10.0		2.5	6.0	15.0	7.5	6.0	45.0	60.0	0.1
10	Acc9	15.0	40.0	50		27.5	6.0	165.0	45	6.0	270.0	435.0	0.4
11	Acc10	40.0	60.0	100		50	6.0	300.0	80	6.0	480.0	780.0	0.7
12	R-check	0.0	0.0	15		0	6.0	0.0	7.5	6.0	45.0	45.0	0.0
13	S-check	100.0	100.0	100		100	6.0	600.0	100	6.0	600.0	1200.0	1.0

Figure 21. Example data set for estimating AUDPC and rAUDPC using MS Excel (S = severity, T = time) (Image source: A Yinka, IITA).

i. Calculate Maximum Potential AUDPC (MPA) by multiplying the total number of days between the first (T1) and next reading (T2) up till the last reading by 100:

First, determine AUDPC as explained earlier for the susceptible check (row 13). Calculate the mean of the first two YAD severity scores of the susceptible statement as shown in Column F, and the time interval between the two observations as shown in Column G. Multiply the two values to obtain AUDPC1 as shown in Column H. Repeat the same process to estimate AUDPC2 values (Column K). Add AUDPC1 and AUDPC2 to get the total AUDPC score (Column L).

The maximum potential AUDPC (MPA) for the most susceptible check in the example data was 1200.

ii. To estimate the rAUDPC of the test lines (rows 2 to 11) using the formula,

$$rAUDPC = \frac{AUDPC}{MPA}$$

For example, rAUDPC for Accn1 = 135/1200 = 0.1 as shown in Figure 21.

4. Interpretation based on rAUDPC values

A low rAUDPC score is an indication that a genotype is resistant or the disease progressed slowly (less susceptible). It is also possible to convert AUDPC or rAUDPC into mean severity comparable to a conventional 1 to 5 severity scoring scale, used for YAD (see Table 2).

5. To estimate mean severity score (S_x) of an accession from AUDPC or rAUDPC, use the formula

$$S_x = S_y \left(\frac{D_x}{D_y} \right)$$

Where S_x is the mean severity scale,

S_y is the maximum YAD severity score of the susceptible check (S-check, row-13 in Fig. 21) as determined by the Estimate (100% in the example given in Fig. 21).

D_x is the AUDPC or rAUDPC of the test accession

D_y is the AUDPC or rAUDPC of the susceptible check

As an example, to convert AUDPC of Acc1,

$$100 \times (135/1200) = 11.25$$

11.25 is within the classification range of 0.1 to 25 (see Table 2) and hence Acc1 can be classified as “Resistant”

S_x values can be calculated using a spreadsheet program like MS Excel (Forbes et al., 2014).

6. Classify the resistance status of the accessions according to the criteria given in Table 2.
7. In conclusion, validate screening for disease resistance by going through the entire process at least twice.

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