

Research Article





Epidemiology of potato late blight disease and other major postharvest biotic stresses in Malawi

Abstract

The epidemiology of Potato Foliar Late Blight (PFLB) disease (Phytophthora infestans) was quantified in major potato production areas of Malawi. Seed multiplication fields, tests clones and local farmers' fields were sampled basing on Area Under Disease Progress Curve (AUDPC). The determined severity values were transformed into AUDPC coefficients characterizing rate of disease spreading across the crop. Results indicate minimum and maximum AUDPC values of 0 and 1050 respectively, with an average value of 233.57. The results show significant statistical differences in PLB disease across seed multiplication fields, test clones and local farmers' fields. AUDPC values differed significantly (p < 0.001) among potato growing districts, as well as sources of seed (aeroponics, sandponics, and vendors). Post-harvest survey targeting potato tubers showed that tubers that were sampled from Mzimba district had the highest likelihood of being infected with Potato Tuber Late Blight (PTLB), followed by potatoes that were sampled from Lilongwe (coefficients, b=1.89, t = 6.11, p-value< 0.001) while the potatoes tubers that were sampled from Ntcheu did not vary in the severity with those that were sampled in Dedza. Susceptibility to potato PTLB among potato varieties were varied, with Rosita likelihood to PTLB disease, while there were no other significant differences to PTLB in the rest varieties (b=1.12, t=4.23, p-value<0.001). An extended study on bacterial wilt (PBW) revealed that disease was influenced by the district where the tubers were sampled ($x^2 = 9.26$, p-value < 0.001) while the type of variety sampled did not have any significant difference on PBW ($x^2 = 3.59$, p-value = 0.268). The presence of potato tuber moth which varied among the sampled districts, was not influenced by variety sampled. The paper has documented and quantified increasing epidemic spread of late blight disease and the consequent effect on sustainable potato production and clean seed systems in Malawi.

Keywords: epidemiology, area under disease progress curve, disease management, potato, Malawi

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Introduction

Potato (Solanum tuberosum L.) is the third most consumed crops, after wheat and rice world-wide.1. However, potato plants are susceptible to many pests and pathogens that seriously limit its production. Potato late blight, caused by the oomycete Phytophthora infestans, is one of the main diseases in potato production.^{2,3} Phytophthora in Greek means 'plant destroyer' which is an apt name for this aggressive pathogen. A historical catastrophe that shows the devastating effects of this disease occurred in Europe in the midnineteenth century, 4,5 Potatoes originated in South-America and were introduced to Europe in the 16th century, without late blight. Since then potato had become the most important crop in Europe, still free of late blight.⁴ Around 1845 a strain of *P. infestans* arrived in Europe, which resulted in severe late blight outbreak, especially in Ireland, where about a third of the population relied on the crop for food, and the impact was severe. The late blight epidemic led to the 'Irish potato famine' during which one million people died because of starvation and another million emigrated to Britain, the U.S. and Canada. 5,6

Potato late blight disease causes annual losses of several billion dollars and it is a global threat for potato growers. The pathogen originated from Central Mexico. In the middle of the 19th century the pathogen was introduced into the US and Europe, Phytophthora infestans is a hemi-biotrophic filamentous fungus-like heterothallic oomycete that attacks living parts of plants from the family Solanaceae. The pathogen causes lesions with necrotic cells in the middle, surrounded by a ring of gradually necrotizing tissue. Once infected,

plants initially appear healthy, before necrotic lesions develop. Under favorable weather conditions, the pathogen can destroy potato foliage in 10 to 15 days and potential yield can be reduced by 50 to 70%.⁷

The Department of Agricultural Research Services (DARS) and the International Potato Centre (CIP) in Malawi have introduced population B3-clones as a way of fighting the disease in Malawi. Population B3 is the most advanced source of horizontal resistance available at CIP to deal with the potato late blight disease other than the transgenic lines. In this population, testing and selection is for horizontal resistance to late blight, 9,10 unlike those previously applied to population A clones, which had a focus on dominant R-genes. Despite these efforts high incidences of late blight continue to be observed. Studies have shown that LB incidences has been ranging from 15% to 25% in Dedza and Ntcheu in on-station evaluation sites. However, there has been no documented survey covering the magnitude of the problem in farmers' fields other than work done in experimental trials.

Disease severity in a plant-pathosystem can be assessed either at the peak of the epidemic or several times at some intervals starting from disease initiation until the end of the epidemic. The former method of assessment measures the cumulative effects of all the factors operating during the course of epidemic viz. the terminal disease severity scores (TDS), while the latter can be used to estimate different parameters like the area under the disease progress curves (AUDPC), relative area under the disease progress curve (RAUDPC), logistic and Gompertz apparent infection rates, the time required



for the disease to reach a specific level of severity in logistic and Gompertz models. Among these parameters, AUDPC has been widely used among others in assessment of partial or quantitative resistance.^{2,3,7,12–14} In this study we;

- (a) Deployed a two data points on the disease progress curve first established by Mukherjee¹⁵ to quantify Potato Foliar Late Blight (PFLB) epidemiology in Malawi;
- (b) Studied post-harvest late blight disease presence in potato tubers herein referred to as Potato Tuber Late Blight (PTLB);
- and (c) Assessed the infestation of Potato Tuber moth (PTM) (*Phthorimaena operculella*), a major pest of potato crops worldwide. The rationale was to establish the effect of these major potato biotic stresses on sustainable potato production in Malawi.

Materials and methods

Study I: Potato Foliar Late Blight Disease survey

Survey and data collection

Potato Foliar Late Blight disease survey was conducted between March and May, 2020 in Dedza, Ntcheu, Thyolo, Ntchisi and Mzimba districts. Potato fields that less than two weeks old were targeted on first survey. The second survey involved visiting the same fields after one month to assess the temporal spread of the epidemic over time. A total of 1794 plants were sampled from the 5 districts. Twenty six plants were assessed per field following an 'X' transect way of assessing disease epidemic 15,16 (13 plants in each way). Disease rating scale of $0-9^{17}$ was used as detailed in Table 1 below.

Table I Disease rating score for Potato LB used in the study

Rating	Symptoms for whole plant assay
0	No visible symptoms apparent
1	Few minute lesions to about 3% of the total leaf area is blighted.
2	3% of foliage affected
3	10% foliage affected
4	25% foliage affected
5	50% of the total plant infected
6	More than 50% but less than 75% stem and foliage affected
7	More than 75% but less than 90% affected
8	Only very few green areas of stem and leaf not affected (much less than 10%).
9	100% foliage completely destroyed and plant died

Mathematical theory

Disease severity was recorded on two points, i.e. the initial stage of disease epidemic and final epidemic assessment, based on the integration of disease progress models modified by Mukherjee suggested that for any real continuous function y = f(t), y > 0, the area under the function is simply the definite integral evaluated between the limit of integration t_0 and T, where $T > t_0$

Suppose
$$y = f(t) = 1/(1 + Ae^{-rt})$$

i.e f(t) is the logistic function with $A = (1 - y_0)/y_0$, where y is the amount of disease in the numerical scale of 0 - 9, y_0 is the value y at $t_0 = 0$, and r is the logistic rate parameter. The area under the disease progress (AUDPC) described by the equation above is then;

AUDPC = $\int \left\{ dt / (1 + Ae^{-rt}) \right\}$ which is evaluated between the limits t = 0 aand T, and substituting in y.

$$= T + \frac{\left[\ln\{(y_0/y_T)\}\right]}{\left[\ln\{y_T/1 - y_T\} - \ln\{y_0/1 - y_0\}\right]/T}$$

Where, y_T is the value of f(t) at = T. Thus, only two assessments of disease are necessary, one at the start of an epidemic and the other at the end of the epidemic or at a critical growth stage. The apparent infection rates in the logistic (r) model is estimated as the regression coefficients (b) of the logit x.

$$r_{TP} = \left[\log_e \left\{ \frac{X_2}{(1 - X_2)} \right\} - \log_e \left\{ \frac{X_1}{(1 - X_1)} \right\} \right] / (t_2 - t_1)$$

Where, X_2 and X_1 are disease severity scores on the first and last day of observation, and t_1 and t_2 are the initial and final day of observation.

Statistical assumptions

There are three conditions that must be fulfilled for a disease epidemic to be modelled using AUDPC.18 The first qualification is that disease resistance must be expressed in terms of a rate parameter rather than in terms of the asymptotic level of disease. This condition was fulfilled in the present study by terminating the disease scoring and AUDPC calculation immediately after severity in the susceptible spreader rows was rated 100% and the plants succumbed to the disease. This made it possible to calculate the Logistic Apparent Infection rates for better comparisons. The second qualification is the period of time over which the disease was present in the crop should be the same for each of the host genotypes. This was also achieved in the present investigation. Conducive environmental conditions favored disease development; disease was established quickly and reached 100% severity within a very short period. Third, anomalous results might occur if disease progress is not continuous and cannot be described by a sigmoid curve. In the present investigation, all the disease progress curves were continuous and more or less sigmoid.

Disease progress in time

Disease severity scores (0-9) on each plant were converted into percentages of foliage tissue blighted. Severity data for each plot were calculated from the resulting percentages. AUDPC values were calculated in R statistical software using agricolae; statistical procedures for agricultural research package.²⁰ Further computations were done in STATA statistical software.

Statistical analysis

One-way Analysis of variance (ANOVA) was done to see if AUDPC values differs across the source of seed (vendor, sandponic and aeroponics) as well as the nature of the production (seed multiplication, test clones and farmers' field). Regression analysis was also done to quantify the differences in disease epidemic spread for both sources of seed and nature of production.

Study 2: Post-harvest survey (Potato Tuber Blight, Bacterial Wilt and Potato Tuber Moth)

To assess post-harvest effect of late blight disease of potato, another survey was conducted in 2021, to look at PTLB incidence as it is a critical stage in the late blight disease cycle, resulting in postharvest yield loss and serving as a source of inoculum. A total of 2000 tubers were assessed from 100 farmer fields. Taking into consideration of the discrete nature of the data, generalized linear model (GLM) fitting a quasi-binomial and binomial distribution was preferred. Prior to running the GLM fitting quasi-binomial family, the potato late blight and bacterial wilt data was transformed to fit in a binary range of 0 and 1. The data transformation involved taking the disease score (1-5) subtracting each score by one and then dividing the outcome by four. This resulted into change of score of one (which was given to a tuber that was clean) to zero, whereas the severely disease score five turns to one.

Transformed score =
$$\frac{(disease score - 1)}{4}$$

Potato tuber moth absence or presence data was fitted to a binomial family, as it was already in binary form where absence was zero and presence was one. After building model for each of the observed pest and diseases, models were tested for collinearity using variance of inflation factor (VIF) and tolerance. The model residuals were also standardized in order to test for possible outliers and influential cases

that were assessed using Cook's distance and hat values (leverage). Each of the estimate (coefficient) models' logistic regression were then converted into odds ratio with 95% confidence interval, its associated probability and R^2 measure on how well the predictors explains the variance in pests and disease severity have been presented in results section. All three models should that the predictors were not collinear and also all the outliers had no major influence on the bias of the model thus increasing the statistical precision. 21

Results and discussion

Potato Foliar Late Blight

Out of the 1,794 sampled plants, the source of seed of 650 were from vendors while 650 and 494 were from aeroponics and sandponics respectively at Bvumbwe Research Station (Thyolo). While both aeroponic and sandponics used tissue culture plantlets, the aeroponic seed production is soiless. Out of the 650 plants from vendors, 260 of them were from Dedza, 130 from Ntchisi and 260 from Ntcheu. Disease severity percentage ranged from 0 to 100 for the initial assessment of the disease, with a mean severity percentage of 57. The final assessment had a mean percentage of 32.79, with a minimum of 0 and maximum of 100. This means that the disease epidemic spread percentage moved from 0.57 to 32.79 after one month. Out of 1794 sampled plants, 1517 did not exhibit any disease reaction during the first assessment whilst during the final assessment, only 80 of them did not show any sign of disease (Table 2).

Table 2 AUDPC values for the potato sampled districts in Malawi

AUDPC	Dedza	Mzimba	Ntcheu	Ntchisi	Thyolo	Total
0	0	19	0	0	61	80
7	0	3	0	0	1	4
14	0	30	0	1	85	116
21	0	257	0	9	104	370
28	0	1	0	0	0	1
35	I	6	0	1	0	8
42	0	0	0	1	0	1
70	7	124	1	25	115	272
84	1	8	0	3	0	12
91	1	2	0	0	0	3
175	19	11	9	26	124	189
189	17	6	0	0	0	23
196	4	0	0	1	0	5
245	2	1	0	5	0	8
350	29	2	33	25	81	170
364	15	5	2	2	0	24
371	5	6	2	0	0	13
385	27	2	32	10	21	92
392	5	0	0	0	0	5
399	18	2	0	0	0	20
406	8	1	4	2	0	15

Table Continued...

AUDPC	Dedza	Mzimba	Ntcheu	Ntchisi	Thyolo	Total
420	6	2	8	3	8	27
434	I	0	0	0	0	1
441	I	0	0	0	0	1
455	4	0	0	0	6	10
490	0	0	0	0	4	4
525	0	0	0	1	0	1
560	4	0	7	2	12	25
574	3	0	0	0	0	3
581	3	0	0	0	0	3
595	27	0	51	5	11	94
609	15	0	3	3	0	21
616	4	2	5	0	0	11
665	4	2	53	0	11	70
672	1	0	0	0	0	1
679	13	0	6	0	0	19
686	2	0	I	5	0	8
700	6	0	18	0	6	30
714	2	1	11	0	0	14
721	2	0	6	0	0	8
735	0	1	2	0	0	3
770	3	0	4	0	0	7
840	0	0	I	0	0	1
1050	0	0	I	0	0	1
Total	260	494	260	130	650	1794

Table 3 shows coefficients on how much of the difference in LB disease for the other two types of sources of seed (aeroponics and sandponic) compared to seed from vendors. The results showed that the mean value of AUDPC values differs significantly (p < 0.001) across the sources of plant material, which means that at least one of the sources of seed is different from the others. Similarly, the results showed that the mean values of AUDPC differs significantly (p < 0.001) across the nature of production. The regression of ANOVA

results of source of seed and AUDPC values shows coefficients on how much of the difference in disease it is for the other two types of sources of seed (aeroponics and sandponics) compared to seed from vendors. The results show that seed from sandponic showed to have a lower AUDPC values than seed from vendors (-374.524). Similarly seed from aeroponic system showed to have lower AUDPC values that seed from vendors (-282.70).

Table 3 ANOVA results and Regression Analysis among the source of seed

Source	Sum of squares	Degree of freedom	Mean squares	Number	of obs = 1,794 Prob > F = 0.0000
Model	46110628	2	23055314		
Residual	51832688	1,791	28940.64		
Total	97943316	1,793	54625.39		
AUDPC	Coefficient	Standard error	т	p> t	Confidence Interval
Sources of see	d				
Vendors (ref)					
Sandponic	-374.55	10.15	-37.28	0	-757.05
Aeroponics	-282.7	9.44	-29.96	0	-565.41
Constant	440.24	6.67	65.98	0	427.15 453.322

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Mean AUDPC values are summarized in Table 2 above. The PFLB disease epidemic spread differed significantly among the districts (p < 0.001). Thyolo district had higher AUDPC values than all the sampled districts while Ntchisi district has lower values. The results further shows that there are statistical differences in PFLB disease across the source of seed (seed multiplication, test clones and farmers' field) as presented in Table 4. In the table source of seed was coded

as 1 = Seed multiplication, 2 = Test clones, and 3 = Farmers' field. The regress table has results for ANOVA and the regression where it shows coefficients on how much of the difference in disease it is for the other two (test clones and farmers' field) compared to seed multiplication. Disease was higher in farmers' field as compared to seed multiplication sites and test clones.

Table 4 ANOVA results and regression analysis among source of seed

Source	Sum of squares	Degree of freedom	Mean squares	Number	of obs = 1,794 Prob > F = 0.0000
Model	46438784	2	23219392		
Residual	51504532	1,791	28757.42		
Total	97943316	1,793	54625.39		
AUDPC	Coefficient	Standard error	т	p> t	Confidence Interval
Source of seed					
Test clones	-110.45	10.98	-10.05	0	-220.91
Farmers' field	291.45	8.94	32.6	0	273.91 308.98
Constant	148.79	5.97	24.91	0	137.07 160.50

Potato Tuber Late Blight, Bacterial Wilt and Potato Tuber moth

A total of 2000 potato tubers of different varieties were sampled in all four districts. Dedza was most sampled district with up to 680 tubers while Lilongwe was the least sampled with 260 tubers. A total of 1221 of potato samples were variety *Violet* and it was most common contributing 61.05% while *Desiree* and *Mwayi* were among the least sampled potato varieties. The results also show that during survey there were variations ($x^2 = 1374.51$, *p-value* = <.001) in variety distribution sampling across the district as some of the potato varieties were not sampled in all districts. Mzimba was the one with most (six) varieties while only one variety (*Violet*) was sampled in Lilongwe. Based on this Dedza district (most sampled district) and *Violet* (most sampled variety) were used as baseline category to assess the likelihood of diseases and pests' severity for subsequent quasi binomial and binomial logistic regression of all other districts and varieties.

Tuber Potato Late Blight (PTLB) severity was assessed using the location (district) and variety sampled. The district from which the potatoes were sampled showed a deviance ($x^2 = 20.05$, p-value < 0.001), indicating that PTLB severity was different among the sampled districts. Variety been sampled also showed significant deviance ($x^2 = 9.81$, p-value < 0.001), indicating that despite the location been sampled some varieties were more susceptible to TPLB than others (Table 6 below). Thus, district where the potato was sourced and variety sampled had significant influence on prevalence of late blight in potato. ($x^2 = 276.13$, p-value < 0.001).

The results show that there were significant differences in disease severity at district level, where potato tubers sampled from Lilongwe and Mzimba had higher likelihood (odds ratio 4.69 and 3.86 respectively, p-value < 0.001) to late blight attack as compared to Dedza (baseline). This may entail that among the potato sampled districts, potatoes that are grown in Mzimba and Lilongwe may be more susceptible to TPLB. On the other hand, the disease susceptibility maybe the same in tubers from Ntcheu and Dedza (Table 5).

Table 5 Likelihood of PTLB among the districts and variety sampled

	Odds ratio	95% CI	P-value
Districts			
Lilongwe	4.69	2.71 – 8.33	<0.001
Mzimba	3.83	2.40 - 6.37	<0.001
Ntcheu	1.15	0.62 - 2.13	0.654
Varieties			
Rosita	1.34	0.89 - 1.99	0.152
Chuma	1.15	0.32 -3.13	0.803
Zikomo	1.01	0.29 - 2.53	0.992
Thandizo	1.49	0.49 – 3.54	0.423
Mwayi	2.28	0.80 – 5.31	0.082
Disiree	0	0.00 - 105.50	0.977

 $\rm R^2$ measures Hosmer and lemmeshow (R_L^2) = 0.078, Cox and Snell = 0.012, Nagelkerke = 0.083

Susceptibility to TPLB among potato varieties were varied; with var Rosita (b=1.12, t=4.23, p-value<0.001) having the highest likelihood to TPLB while there were no other significant differences in the disease in the rest of the varieties. The Hosmer and Lemmeshow (R_L^2) indicates that the model only explains 10.0% while Cox and Snell shows that the model explains 1.5% and Nagelkerke indicates that the model is able to explain 10.7% of TPLB disease severity (Table 5).

The results show that seed from sandponic had a lower AUDPC values than seed from vendors (-374.524). Similarly seed from aeroponic system showed to have lower AUDPC values that seed from vendors (-282.70). This is not surprising as seed from both aeroponic and sandponic systems originated from tissue culture and hence low chances to be infected. Studies elsewhere has shown that LB disease can enter the plant system through poor seed systems.^{2,22} In

Malawi performance of aeroponics seed system as a way to mitigate and control major potato diseases has already been assessed and documented.²³ Thus, there is need to strengthen potato seed system so as to achieve clean systems.

Potato Late Blight disease was higher in farmers' fields as compared to seed multiplication sites and test clones. This is not surprising since most farmers use seed from vendors which in most cases isn't absolutely clean seed. Furthermore, seed multiplication had improved varieties that have been officially released for production in Malawi and have a background of the durable LB resistance. Most of these genotypes are tolerant to late blight and other major potato pathogens.¹¹ Farmers need to be trained on proper seed systems to avoid use of seed from vendors. Small-scale seed plot technique which includes Low cost Diffuse Light Store, non-diseased tubers, soil amendments and less susceptible cultivars have been important components for integrated management of LB disease.^{24,25}

The study has also documented statistical variations in LB presence among the sampled districts with Thyolo district having higher AUDPC values than all the sampled districts while Ntchisi district has lower values. The plants sampled in Thyolo were predominantly test clones at Byumbwe Agricultural Research Station. During variety evaluations, test clones are seldomly sprayed for late blight to test their resistance to the limit, hence this higher disease epidemics as compared to plants that were sampled in other districts since they were released varieties and were under LB control. Furthermore, the seed at the research stations are normally recycled for 4 or 5 seasons and therefore, accumulating diseases.²⁶ The low values in Ntchisi could also be attributed that there were generally few fields sampled in that district than other districts. Of much interest is Ntcheu and Dedza which are predominantly the major potato growing areas and all fields sampled there were local farmers' fields. These districts present a factual status of LB disease epidemic spread in local farmers' fields in Malawi.

The study also established that LB disease was higher in farmers' fields as compared to seed multiplication sites and test clones. This is not surprising as well since most farmers use seed from vendors which in most cases isn't absolutely clean seed. Furthermore, seed multiplication had improved varieties that have been officially released for production in Malawi. Most of these genotypes are tolerant to late blight and other major potato pathogens. Farmers need to be trained on proper seed systems to avoid use of seed from vendors. Small-scale seed plot technique which includes Low cost Diffuse Light Store, non-diseased tubers, soil amendments and less susceptible cultivars have been important components for integrated management of LB disease.^{24,25}

Potato bacterial wilt and potato tuber moth

Lastly, the sampling and assessment of potato tubers revealed continuing threat of PTLB to seed system. The model showed a significant improvement on trying to explain the occurrence of potato Bacterial wilt (PBW). This significance was influenced by the district where the tubers were sampled, which a deviance with ($x^2 = 9.26$, p-value < 0.001. On the other hand, the model showed that the type of variety sampled did not have any significant difference on PBW ($x^2 = 3.59$, p-value = 0.268).

Results show that occurrence of PBW was significantly lower on potato tubers sampled in Mzimba (b= -1.02, t=-3.35, p-value <0.001), seconded by potato sampled in Ntcheu (b=-1.052, t=-3.0, p-value=0.003) as shown in Table 7 above. However, there was no significant difference in the occurrence of BW disease on potatoes

that were sampled in Dedza and Lilongwe. This may imply that there is high likelihood of potato BW occurrence to potato grown in Dedza and Lilongwe as compared to those grown in Mzimba and Ntcheu. The Hosmer and Lemmeshow (R_L^2) indicates that the model was able to explain 5.7% of potato late blight severity while Cox and Snell show's that the model explains only 0.6% and Nagelkerke's indicates that the model explains 6% of the variations. The presence of PTM varied among the sampled districts ($x^2 = 50.69$, p-value < 0.001) while there were no significant variations among its infestation in sampled varieties ($x^2 = 9.07$, p-value = 0.142). The PTM tuber moth infestation was significantly higher in Mzimba (b = 1.98, z = 4.62, p-value <0.001). However, there is high likelihood that presence of PTM was not influenced by variety sampled as indicated by Model R2 measure indicates that it explains measures 10.1 % Hosmer and Lemmeshow (R_L^2), 3.0% Cox and Snell and 12.0% Nagelkerke. It is not surprising that potato tubers that were sampled from Mzimba and Lilongwe had the highest likelihood of been infected with TPLB disease. These are upcoming potato growing areas where farmers have limited knowledge of major potato pests and diseases.

Conclusions

The surveys have documented continued threat of potato late blight disease to sustainable potato production in Malawi. The disease continues to occur in epiphytotic proportion throughout the country. This study has quantified and documented the first comprehensive results combining foliar late blight as well as tuber late blight as major biotic challenges in potato sub sector in the country. The survey has also linked the disease pressure, incidences, and severity to source of seed (including test clones), as well as varieties involved so that we have an in-depth understanding of the epidemic from breeding trials to farmers fields and seed multiplication sites. Seed sourced from local vendors as well as potato farms for local farmers has highest disease epidemic spread. Thus, there is need for intensifying integrated disease control and management. It was further noted that even potato tubers stored as seed for subsequent planting season had substantial levels of LB infection, implying that some farmers continue to use seed that is not clean. Our farmers need proper seed systems so they store their own clean seed and not always rely from local vendors for seed purchase.

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Conflicts of interest

The authors declare that there was no conflict of interest.

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