Studentship Project: Annual Progress Report January/2020 to April/2021

Student Name:	Alicia A. Farmer	**AHDB Project Number:**	CP 186
Project Title:	Understanding populations of the lettuce downy mildew pathogen *Bremia lactucae* to inform integrated disease management		
Lead Partner:	James Hutton Institute and University of St. Andrews		
Supervisor:	Dr Alison Lees, Dr David Cooke, Dr Tim Pettitt, Prof John Jones		
Start Date:	27 Jan 2020	**End Date:**	27 April 2023

1. Project aims and objectives

Lettuce downy mildew (LDM) is a foliar disease caused by *Bremia lactucae*, that results in reduced quality, yield and marketability of lettuce crops. This disease is more prevalent in cooler climates, such as the U.K. and is currently managed through a combination of routinely sprayed fungicides and host resistance (Barriere et al., 2014, Fall et al., 2016).

As lettuce crop value is closely related to visual appearance and LDM epidemics establish rapidly after first symptom appearance, prophylactic fungicide sprays are applied regularly regardless of pathogen presence in-field. This indiscriminate approach, along with the use of monoculture and successive plantings, has increased the risk of evolution of fungicide insensitivity and new virulences within *B. lactucae* populations (Crute et al., 1987; Crute and Harrison, 1988; Brown et al., 2004; Parra et al., 2016).

IPM strategies, especially cultivar selection along with targeted chemical controls and lettuce breeding.

The project also aims to validate a diagnostic LAMP (Loop-mediated Isothermal Amplification) assay for in-field spore detection, to determine risk thresholds and to analyse whether the LAMP ass

According to Crandall et al. (2018), early detection is crucial in the management of aerial oomycetes such as *B. lactucae,* along with an understanding of the contemporary pathogen population: how they migrate, and their phenotypic characteristics. Understanding the population diversity both genotypically and phenotypically would allow for more informed decisions on crop breeding along with fungicide and resistance gene stewardship.

The main aim of the project is to understand the population diversity of *B. lactucae* to help inform integrated pest management (IPM) of LDM. To achieve this aim I will examine the pathogen population with simple sequence repeat molecular markers (SSRs) to genotypically profile isolates. SSR markers are not diagnostic for specific traits but are a commonly utilised method to get a representation of population diversity. We hypothesise that isolates belonging to a common genotye profile will be more phenotypically similar than those with a different genotype. Therefore we aim to explore associations between genotype and phenotype and factors including host cultivar, aggressiveness, fungicide sensitivity and location. This information will help inform growers to allow better targeted IPM strategies when LDM is encountered. Profiling the contemporary pathogen population of *B. lactucae* will provide information to help inform ay has utility in providing early warning of infection to growers for IPM strategies. Knowledge of inoculum presence and risk thresholds can provide decision support for the timing and use of chemical control.

2. Key messages emerging from the project

- ***B. lactucae* genotypes:** Molecular (SSR) markers are being used to analyse the genomic diversity of the *B. lactucae* population. Results to date show that ten candidate SSR markers used in combination have the potential to distinguish between different *B. lactucae* strains.
- **Phenotype characteristics:** The small number of 2020 UK *B. lactucae* samples available were race-tested using the IBEB differential assay and showed a similar virulence profile to European race Bl:24 EU. The UK isolates tested were unable to overcome resistance (*Dm*) genes *Dm3, Dm14, Dm15,* and *Rsal-1*.
- **LAMP diagnostic assay:** Due to low incidence of LDM in 2020 (weather not conducive to disease) results from spore trapping and detection were inconclusive. However, simulations of field trapping demonstrated that the diagnostic LAMP assay was more robust than the conventional qPCR assay for detecting *B. lactucae* spores from field samples spiked with *B. lactucae* spores.

3. Summary of results from the reporting year

SSR Markers

Simple sequence repeats (SSR) were chosen to analyse the genomic diversity of the *B. lactucae* population, as they are an accepted way of discriminating genotypes based on length variation (alleles) at a range of points in the pathogen genome (loci) (Cooke and Lees, 2004; Li et al., 2013). A combination of different markers with different allele lengths can create a unique genotypic profile for an isolate, essentially giving a 'fingerprint'.

To date, we have been testing 10 candidate SSR markers (10 loci), which were obtained from collaboration with UC Davis (USA). These 10 SSR markers were tested on two UK samples (2020_Bl2.1 and 2020_Bl4E) and two reference races (NAK_Bl1 and NAK_Bl36 obtained from Naktuinbouw, Netherlands) to check if they could distinguish between them. The reference races differed at seven loci and by nine loci to the UK samples (Table 1). The UK samples had almost identical alleles, although the dataset tested so far has been limited by availability of samples. A further 40 candidate SSR markers have been identified for use if these markers do not discriminate between UK samples. The test is being optimised into one reaction (currently 10 reactions are performed) before being used on other *B. lactucae* DNA samples to avoid using up DNA sample material.

Heterozygous loci were observed in both UK and reference races, with some UK samples having more than two alleles observed at a locus, for example NAK_Bl36 at locus Marker_7. The difference in number of alleles observed was expected to be one-two as the European *B. lactucae* population has been reported to be preferentially sexually reproducing and diploid. Populations that do not rely on sexual reproduction have higher rates of heterokaryons (mycelium that can contain multiple genetically diverse nuclei) and therefore more than two alleles can be expected to be observed. This is not expected to occur frequently in the UK Bremia population.

Table 1 Genotypic profiles of* B. lactucae *samples tested in 2020. *Each row is a different* B. lactucae *sample, which was tested in duplicate, and each column is an SSR marker (locus), the different colours at each marker represent a different length/allele. The top two rows are the UK samples collected from 2020 (Bl4E and Bl2.1), below are two reference races NAK_16 (Bl:16EU) and NAK_36 (Bl:36EU).*

Sample Name		Marker_1				Marker_2			Marker_3			Marker_4			Marker_5			Marker_6			Marker_7			Marker_8			Marker_9			Marker_10		
		Allele 1	Allele 2	Allele 3	Allele 4	Allele 1	Allele 2	Allele 3	Allele 1	Allele 2	Allele 3	Allele 1	Allele 2	Allele 3	Allele 1	Allele 2	Allele 3	Allele 1	Allele 2	Allele 3	Allele 1	Allele 2	Allele 3	Allele 1	Allele 2	Allele 3	Allele 1	Allele 2	Allele 3	Allele 1	Allele 2	Allele 3
2020_Bl4E	R1	150	156			242	278		233	233		284	286		170	170		235	244		288	300		124	130		233	233		287	287	
	R2	147	150	153	156	242	278		233	233		284	286		170	170		235	244		288	300		124	130		233	233		287	287	
2020_Bl2.1	R1	150	156			242	278		233	233		284	286		170	170		235	244		288	300		130	130		233	233		287	287	
	R2	150	156			242	278		233	233		284	286		170	170		235	244		288	300		124	130		233	233		287	287	
NAK_16	R1	150	150			242	284		233	233		284	286		168	170		235	238		278	288		124	130		220	233		284	287	
	R2	150	150			284	284		233	233		284	286		168	170		235	238		278	288		124	130		220	233		284	287	
NAK_36	R1	150	156			284	284		219	233		284	294		170	170		235	238		231	282	288	124	130		220	233		284	284	
	R2	150	156			284	284		219	233		284	294		170	170		235	238		231	282	288	122	130		220	233		284	284	

Phenotypic characteristics:

Race testing was carried out on UK *B. lactucae* samples collected in 2020, and the two reference races (NAK_16 and NAK_36) using a differential assay. The differential assay is a standardised method for race determination set by the International Bremia Evaluation Board (IBEB) and is comprised of a standard set of lettuce cultivars with known resistance genes (*Dm*) that are inoculated with the test *B. lactucae* sample to determine which *Dm* genes it can overcome as indicated by successful infection. Infection scoring is translated into a sextet code that is used to represent the race of *B. lactucae*. Race / virulence testing was done following the IBEB protocol, using seeds supplied by Naktuinbouw (NAK, Netherlands).

The virulence assay was successfully completed for the three UK *B. lactucae* samples collected in 2020 (Table 2), with infection of the universally susceptible control (Cv. Green Towers) by each *B. lactucae* sample, and no obvious contamination. None of the 2020 UK *B. lactucae* samples infected the differential lettuce cultivar Dandie, which contains the *Dm3* resistance gene. This was in accordance with previous tests conducted on UK isolates in 2019 (data not shown). In IBEB race testing records from other countries this cultivar was commonly infected. *B. lactucae* samples 2020_Bl2 and 2020_Bl4E had the same virulence profile, which was different from previously scored isolates. The profile for sample 2020_Bl4G was similar to the other two UK *B. lactucae* samples with the only difference being a negative score for infection of the cultivar Design. When checking the UK *B. lactucae* samples against prior IBEB race virulence profiles, sample 2020_Bl4G's profile matched with the European race Bl:24EU. Overall, there are a few *Dm* genes and cultivars that the *B. lactucae* samples collected in 2020 were not able to successfully infect:

- **R genes:** *Dm3, Dm14, Dm15, and Rsal-1*
- **Cultivars:** Dandie, UC DM14, NumDm15, FrRsal-1, RYZ 2164, RYZ 910457, Bedford, Balesta, Bartoli, and Kibrille

It should be noted that even though IBEB scorings are based on binary positive '+' and negative '-' sporulation results, occasionally there are cases where the sporulation is very weak in comparison to the susceptible seedling, or there is necrosis without sporulation, these are denoted by '(-)', and this is counted as a negative score. With the IBEB scoring system in mind, and as the official virulence score for the *B. lactucae* race Bl:24 EU scored '(-)' for the cultivar Design, there is a chance that all UK *B. lactucae* samples had the same virulence profile, especially as 2020_Bl4E and 2020_Bl4G were from the same outbreak. Although possible evolution of virulence in 2020_Bl4G should not be discounted. The virulence profile of the NAK reference races, NAK_Bl16 and NAK_Bl36, in our experiment were more complex than the official IBEB race virulence scoring, (Bl:16 EU and Bl:36 EU respectively). NAK_Bl36 successfully infected three additional cultivars, and NAK_Bl16 successfully infected two additional cultivars, than their respective IBEB race virulence profiles would suggest. Despite the harmonisation of scoring, there is still interpretation involved that can lead to different virulence scores and race determination**.**

An interesting observation to note is that the UK *B. lactucae* samples had similar virulence profiles and also a similar genotypic profile in the case of 2020_Bl2 and 2020_Bl4E. This might suggest that they were the same strain (clonal), despite being isolated in two different non-adjacent counties (West Sussex and Cambridgeshire). Whilst genotypic differences might be anticipated in such widely dispersed samples, other parameters not determined such as source of seeds or lettuce plugs, or possibly even weather conditions may explain the close similarity of these two isolates. Nevertheless, it is important to note that this is preliminary data, and we hope to build a larger data set for more robust conclusions, and therefore it is of utmost priority to obtain further live LDM samples to have the viable spores to test phenotypic parameters to better investigate and understand any associations between genotype and phenotype.

Table 2 *Differential test on 2020* B. lactucae *isolates, two reference races (NAK_Bl16 and NAK_Bl36) compared with three IBEB scored races. Legend: '+' is susceptible,'-' is resistant, '(-)' indicates symptoms of necrosis but no sporulation or very weak sporulation, as defined in the harmonized scale given in IBEB scoring scale.*

Cultivar	Green Towers	Dandie	R4T57 D	UC DM14	NunDm15	CG Dm16	Colorado	FrRsal-1	Argeles	RYZ 2164	RYZ 910457	Bedford	Balesta	Bartoli	Design	Kibrille	C Sextet Code
Resistant gene		Dm3	Dm4	Dm14	Dm15	Dm16	Dm18	Rsal-1	R38								
Grid Position		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	
Sextet Value		1	2	4	8	16	32	1	2	4	8	16	32	1	2	4	
2020_Bl2	+	-	+	-	-	+	+	-	+	-	-	-	-	-	+	-	50-02-02
2020_Bl4E	+	-	+	-	-	+	+	-	+	-	-	-	-	-	+	-	50-02-02
2020_Bl4G	+	-	+	-	-	+	+	-	+	-	-	-	-	-	-	-	50-02-00
NAK_Bl16	+	+	+	-	-	+	-	-	-	+	+	-	-	-	-	-	19-12-00
NAK_Bl36	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	63-15-07
Bl: 16EU	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	19-00-00
Bl: 24EU	+	-	+	-	-	+	+	-	+	-	-	-	-	-	(-)	-	50-02-00
Bl: 36EU	+	+	+	+	-	+	+	+	+	+	+	-	-	+	(-)	-	55-15-01

4. Summary of results from the reporting year

(rt-)LAMP diagnostic:

The real-time loop-mediated isothermal amplification (rt-LAMP) procedure is a diagnostic assay that can amplify DNA at a constant temperature. The rt-LAMP assay is generally more robust, simple, quicker, and cheaper compared to the conventional real-time quantitative polymerase chain reaction (qPCR) previously developed for *B. lactucae* (Kunjeti et al., 2016). As the LAMP assay does not require a thermocycler like PCR procedures, it can be more readily adapted for use outside the laboratory (Notomi et al., 2015).

This diagnostic test was developed and validated under laboratory conditions as part of the wider AHDB project (CP 184) with which the studentship is aligned. The studentship aim was to assess the procedure's performance under field conditions, determining risk threshold levels, detection levels, and testing sensitivity when exposed to the commercial field environment.

Ultimately, these in-field trials are assessing the utility of the rt-LAMP assay for early detection for input into decision supports (DS) for IPM. Currently, DS are based on meteorological data meaning that sprays may be recommended at times when LDM inoculum is absent or below risk threshold levels. However, if the assumption that aerial spores are the main means of infection and transmission of *B. lactucae* is correct, warnings from an in-field LAMP assay could reduce the number of sprays recommended by an appropriately modified DS.

Field trials:

Field sampling of spores was carried out between 07/07/2020 -09/10/2020 at two commercial farms, one in England (Cambridgeshire) the other in Scotland (Fife). Field samples were collected using two rotorod spore samplers (Burkard) twice a week, with an additional mutli-vial spore sampler (Burkard) at the English site. Spore samplers were moved to follow the field plantings of lettuce, which changed throughout the growing season at both farms.

The rt-LAMP assay initially identified several samples as positive for the detection of *B. lactucae* DNA, though in general these did not amplify in the qPCR. The putative positive samples were checked a second time using both the rt-LAMP and qPCR tests, along with a melt-curve analysis which checks for non-specific amplification. Amplification in real time was measured using cycle thresholds (C_T the point at which the detection exceeds the background noise), and comparing the samples to known amounts of *B. lactucae* DNA standards. The lower the C_T the quicker the rt-LAMP has

detected *B. lactucae* DNA, implying more DNA is present in the sample. With the rt-LAMP assays the higher the C_T the greater the chance that amplification is not specific; a C_T "cut-off" for reliable positive results is often implemented (Optigene, 2018).

Only one field sample, GFMV 29/9 amplified in the rt-LAMP assay (C_T: 34.9 and 38.1), qPCR assay (Average C_T: 49.4)., and had the same melting temperature as the *B. lactucae* DNA standards (Tm = 83.62°C) in melt curve analysis. No robust conclusions can be derived from one positive sample.

Industrial partners mentioned low incidence of LDM in 2020, likely due to the weather patterns of 2020 not being conducive to disease. In the absence of positive field-samples, simulations of the field environment and investigations in the lab based on Lees et al. (2019) protocol were carried out as follows:

1. Artificially adding *B. lactucae* spores to the rotorods used on the rotorod spore samplers, then treating as a field sample. This was done to check sensitivity of the collection method.
2. Spiking field samples with a known amount of *B. lactucae* DNA to test for possible inhibition of the rt-LAMP and qPCR assays - sampling in the field environment can introduce inhibiting or contaminating substances to the sample, which could persist through DNA extraction protocols. Notably some of the DNA extracted from multi-vial spore samplers did occasionally produce dark DNA suspensions, which could interfere with the fluorescent detection used by the rt-LAMP and qPCR assays. A purification method was tested on some of the contaminated samples to check whether it improved readings.

Sampling rotorods with artificially added spores (1):

A serial dilution of spores (1000, 100, 10 and 1 spores/ml) were added to sampling rotorods, which were subsequently treated in the same way as field samples. Each diagnostic assay used a different volume of template DNA therefore varying the amount of template DNA (and spore equivalents) per assay.

The rt-LAMP assay reliably detected template DNA extracted from rotorod samplers spiked with 100 spores of *B. lactucae*, which, accounting for DNA extraction, corresponds to approximately 14 spores (Average C_T = 20.9, SD = 0.6), (Figure 1). The 20.9 C_T for the detection "cut-off" is likely to be too low for field collected samples as the positive in-field sample GFMV 29/9 was detectable at C_T values of 34.9 and 38.1 C_T.

The detection threshold (C_T value) for DNA extracted from 10 spores added to sampling rotorods was approximately C_T = 27.9, (SD= 3.5). Although this is more variable and less reliable (n=3, out of 4) than when 100 spores were added it showed promise for specific amplification (Tm= 83.62°C). Therefore, more work will be done to establish the rt-LAMP assay detection threshold of samples collected by rotorod sampling, by repeating the validation using a wider range of spore dilutions (between 100-10 spores).

The qPCR reliably detected template DNA of *B. lactucae* equivalent to approximately 1 spore per reaction (Figure 2). The standard curve from the qPCR, which was used to calculate the starting concentration of *B. lactucae* template DNA, had a correlation coefficient of R^2= 0.979, this indicated a linear relationship between template DNA concentration and detection, indicating that the assay was providing a good comparison to the rt-LAMP assay.

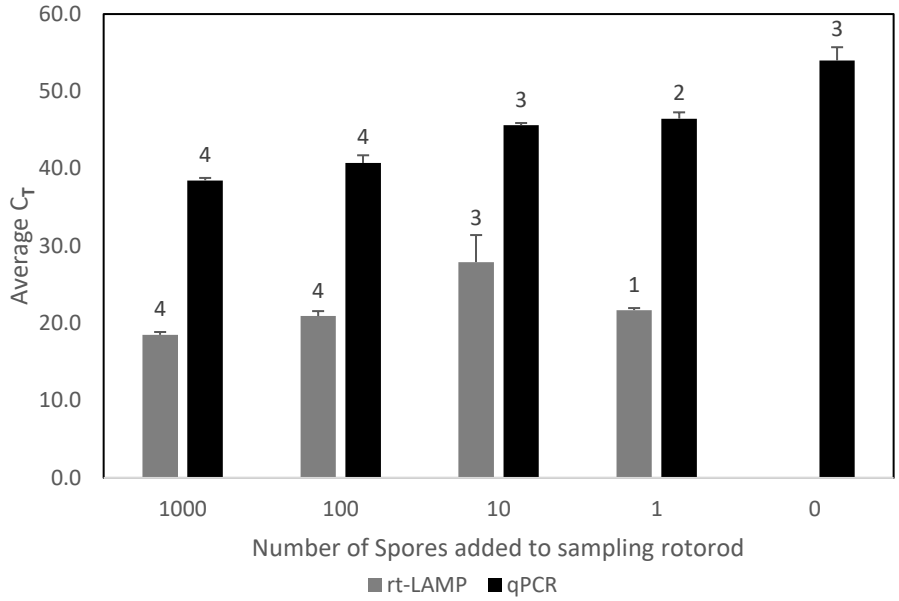

Figure 1 *Mean number of cycles (C_T) taken for the detection of DNA of* B. lactucae *extracted from spores using rt-LAMP and qPCR. DNA extracted from rotorods samplers to which a known number of spores had been added. Error bars are average standard deviation. Data label above columns indicates the number of replicate samples from which* B. lactucae *DNA was detected by rt-LAMP or qPCR (n=4).*

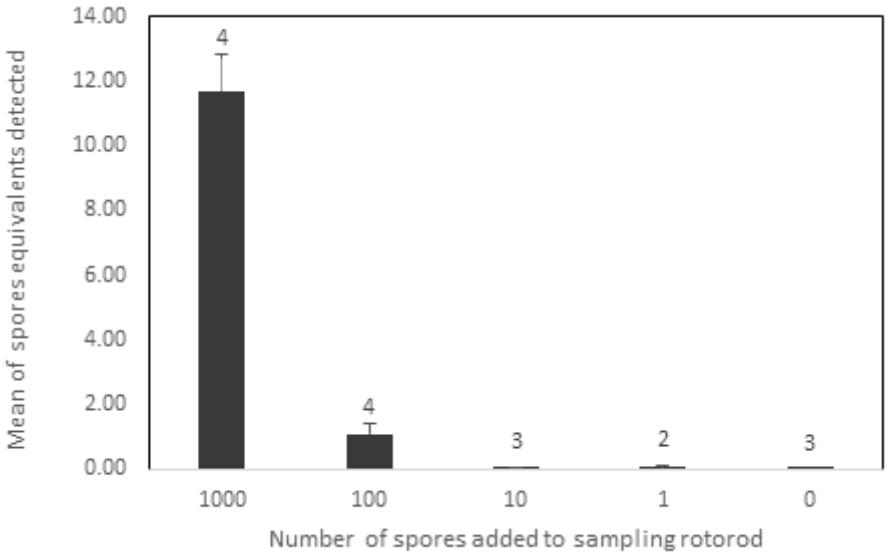

Figure 2 Mean number of B. lactucae *spore equivalents detected using qPCR on DNA extracted from rotorods to which a known number of spores had been added. Error bars are average standard deviation. Data label above columns indicates the number of replicate samples from which* B. lactucae *DNA extracted from spores was detected by qPCR, (n=4).*

Field-samples spiked to test for inhibition to the rt-LAMp and qPCR assays:

Although only a limited number of analyses were possible, the results of the samples spiked with *B. lactucae* DNA indicated that contaminant discolouration of DNA extracted from spore samples has an effect on the detection efficiency of the rt-LAMP and qPCR assays (Figure 3). Purification of contaminated samples improved amplification in comparison to non-purified contaminated. However, this improvement still did not match amplification patterns as the non-contaminated samples, suggesting that collection techniques prone to contamination (multi-vial samplers) should preferably be avoided.

Inhibition was observed more frequently in qPCR than in rt-LAMP assays, fewer uncontaminated samples and no contaminated samples amplified in qPCR in comparison to the rt-LAMP assay (Figure 3). As contaminants such as soil and insects are more abundant in field environments compared to sterile laboratory conditions, these results showing amplification using rt-LAMP being less prone to inhibition by contaminants indicate this technology is potentially more suited for field use.

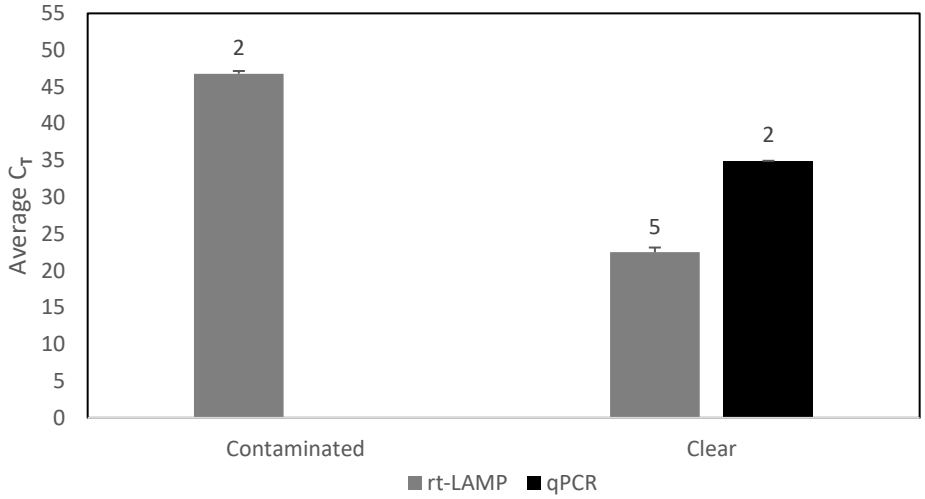

Figure 3 *Mean number of cycles (C_T) taken for the detection of* B. lactucae *DNA using rt-LAMP and qPCR assays on samples spiked to a 1:1 ratio with* B. lactucae *DNA equivalent to 700 spores/µl. Error bars are average standard deviation. Data label above columns indicates replicate number of samples in which* B. lactucae *DNA detected using rt-LAMP or qPCR assays, (n=5).*

5. Key issues to be addressed in the next year (2021/22):

SSRs and phenotype testing:
We will undertake the optimisation needed to allow all the markers to be combined in one reaction, before testing with other *B. lactucae* DNA samples. We will verify that the markers capture population diversity with further tests on a wider selection of *B. lactucae* samples. More samples need to be collected to properly represent the UK population diversity and to monitor traits in the population. Phenotypic characteristics can only be tested if living spores are collected therefore encouraging or collecting leaf samples is of the highest priority. Collaboration with JHI partners in project CP 184 means that fungicide sensitivity information will be collected for 2020 and 2021 isolates to check for association with phenotype.

LAMP diagnostic assay:
The low disease season last year means that the spore sampling at commercial sites will be repeated this year. In the event that another low LDM disease season may occur, inoculated field trials to replicate and model the commercial environment will be set up to validate the diagnostic assay under field-environmental conditions close to its intended use.

6. Outputs relating to the project

(events, press articles, conference posters or presentations, scientific papers):

Output	Detail
AHDB progress meetings	Attended and presented project plans and progress to supervisors, AHDB staff and industry representatives on 17 June 2020 and 1 April 2021
AHDB student conference March 2020	Poster was prepared describing project plans, outlining what the problem was and how I aimed to address it.

AHDB student conference March 2021	A three-minute pre-recorded presentation introducing the project to fellow students, at a level for non-specialist audience, e.g. no use of jargon for molecular biology unless it was explained.
JHI student conference January 2021	A 15-minute presentation focused on the SSR markers and current progress tailored to be understandable to non specialist audience.
G's Fresh Annual Leafy salads Development trials Review November 2020	Brief talk to industry partner on results of the LAMP and SSR markers and how their input contributed.
Virtual Tour of G's Fresh Lettuce production **July 2020**	Introduction and virtual tour of G's Fresh premises and a discussion on how lettuce is produced in a commercial environment. This provided insight on how IPM strategies to manage LDM are implemented in a farming system overall rather than with just focus on the disease.The discussion also touched on how knowledge from research papers are implemented and also provide a focus on gaps in knowledge in the UK system to potentially address in the research project.

7. Partners (if applicable)

Scientific partners	UC Davis (collaborator)
Industry partners	G's Fresh, Kettle Produce,
Government sponsor	

8. References:

BARRIERE, V., LECOMPTE, F., NICOT, P. C., MAISONNEUVE, B., TCHAMITCHIAN, M. & LESCOURRET, F. 2014. Lettuce cropping with less pesticides. A review. *Agronomy for Sustainable Development,* 34**,** 175-198.

BROWN, S., KOIKE, S. T., OCHOA, O. E., LAEMMLEN, F. & MICHELMORE, R. W. 2004. Insensitivity to the fungicide fosetyl-aluminum in California isolates of the lettuce downy mildew pathogen, Bremia lactucae. *Plant Disease,* 88**,** 502-508.

COOKE, D. E. L. & LEES, A. K. 2004. Markers, old and new, for examining Phytophthora infestans diversity. *Plant Pathology,* 53**,** 692-704.

CRANDALL, S. G., RAHMAN, A., QUESADA-OCAMPO, L. M., MARTIN, F. N., BILODEAU, G. J. & MILES, T. D. 2018. Advances in Diagnostics of Downy Mildews: Lessons Learned from Other Oomycetes and Future Challenges. *Plant Disease,* 102**,** 265-275.

CRUTE, I. R. & HARRISON, J. M. 1988. Studies on the Inheritance of Resistance to Metalaxyl in Bremia-Lactucae and on the Stability and Fitness of Field Isolates. *Plant Pathology,* 37**,** 231-250.

CRUTE, I. R., NORWOOD, J. M. & GORDON, P. L. 1987. The Occurrence, Characteristics and Distribution in the United-Kingdom of Resistance to Phenylamide Fungicides in Bremia-Lactucae (Lettuce Downy Mildew). *Plant Pathology,* 36**,** 297-315.

FALL, M. L., VAN DER HEYDEN, H. & CARISSE, O. 2016. A Quantitative Dynamic Simulation of Bremia lactucae Airborne Conidia Concentration above a Lettuce Canopy. *Plos One,* 11.

KUNJETI, S. G., ANCHIETA, A., MARTIN, F. N., CHOI, Y. J., THINES, M., MICHELMORE, R. W., KOIKE, S. T., TSUCHIDA, C., MAHAFFEE, W., SUBBARAO, K. V. & KLOSTEIMAN, S. J. 2016. Detection and Quantification of Bremia lactucae by Spore Trapping and Quantitative PCR. *Phytopathology,* 106**,** 1426-1437.

LEES, A. K., ROBERTS, D. M., LYNOTT, J., SULLIVAN, L. & BRIERLEY, J. L. 2019. Real-Time PCR and LAMP Assays for the Detection of Spores of Alternaria solani and Sporangia of Phytophthora infestans to Inform Disease Risk Forecasting. 103**,** 3172-3180.

LI, Y., COOKE, D. E. L., JACOBSEN, E. & VAN DER LEE, T. 2013. Efficient multiplex simple sequence repeat genotyping of the oomycete plant pathogen Phytophthora infestans. *Journal of microbiological methods,* 92**,** 316–322.

NOTOMI, T., MORI, Y., TOMITA, N. & KANDA, H. 2015. Loop-mediated isothermal amplification (LAMP): principle, features, and future prospects. *Journal of Microbiology,* 53**,** 1-5.

OPTIGENE 2018. Determining the Detection Limits of a LAMP Assay .

PARRA, L., MAISONNEUVE, B., LEBEDA, A., SCHUT, J., CHRISTOPOULOU, M., JEUKEN, M., MCHALE, L., TRUCO, M. J., CRUTE, I. & MICHELMORE, R. 2016. Rationalization of genes for resistance to Bremia lactucae in lettuce. *Euphytica,* 210**,** 309-326.