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# PURE

## Pesticide Use-and-risk Reduction in European farming systems with Integrated Pest Management

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### Collaborative Project SEVENTH FRAMEWORK PROGRAMME

## D11.8

### Evaluation of the realized performance and proof of concept of the 5 technologies developed

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## 1. Summary

Work package 11 developed new technologies for crop protection based on the combination of detection and monitoring methods at the macro scale (i.e. on whole field scale) and micro scale level (i.e. certain positions in the field) with decision engines and innovative crop protection solutions.

Specifically the objectives were:

1. To do airborne sampling and optical sensing methods for macro scale mapping (Task 11.1);
2. To do molecular based diagnostic tools for micro scale identification and monitoring (Task 11.2);
3. To develop new generation of generic decision engine for weed control (Task 11.3);
4. To do mating disruption solutions for pest control in protected vegetables and grapevine (Task 11.4);
5. To work on precise crop protection products application based on canopy density sensing (Task 11.5).

Spore trapping devices have been developed and patented. Good quality spores have been identified on the traps. Suitable wavelengths have been identified that can be used for detection of yellow rust detection in winter wheat with a 99.1% accuracy. However, more data is needed to be able to identify wavelengths that are suitable to detect yellow rust in an early infection stage, in order to meet the requirements for an early warning system.

Real-time TaqMan can be used as a molecular based diagnostic tool for micro scale identification and monitoring of selected pests.

A generic DSS for integrated weed management was developed and tested in several countries and cropping systems. With this DSS the herbicide use can be reduced by 20 to 70% depending on country, crop and weed population. The DSS meets the predefined criteria.

Prototype equipment for vibrational disruption for *Scaphoideus titanus* control was developed and tested under field and laboratory conditions. The trials showed that mating could be disrupted and optimization trials have been performed to be able to save on energy consumption and improve efficacy. Slow release dispensers with *Tuta absoluta* pheromone have been manufactured and field experiments in tomato crops in Italy were performed. Trials were performed in the spring-summer cycle and in the summer-autumn cycle and show good results.

For precision spraying in fruit orchards a prototype sprayer has been constructed and tested in commercial fruit orchards in multiple growing seasons. The sprayer has a laser scanner to measure canopy density, and with that sensor and appropriate algorithms developed canopy density spraying has been made possible. Although driving speed does not meet up with commercially required driving speed, the CDS sprayer will be developed further to meet the driving speed requirements.

During the preparation of the cell sprayer "SprayCell" for field tests in vegetable crops unsolvable software problems were encountered. Therefore, it was decided to stop the work on the SprayCell. An alternative cell sprayer was selected, the "VisionSpray" which was tested in the field in 2014. Currently, data is being analysed.

## 2. Objectives

WP 11 aimed at developing new technologies for crop protection based on the combination of detection and monitoring methods at the macro scale (i.e. on whole field scale) and micro scale level (i.e. certain positions in the field) with decision engines and innovative crop protection solutions.

Specifically the objectives were:

1. To do airborne sampling and optical sensing methods for macro scale mapping (Task 11.1);
2. To do molecular based diagnostic tools for micro scale identification and monitoring (Task 11.2);
3. To develop new generation of generic decision engine for weed control (Task 11.3);
4. To do mating disruption solutions for pest control in protected vegetables and grapevine (Task 11.4);
5. To work on precise crop protection products application based on canopy density sensing (Task 11.5).

### 3. Approach

#### 3.1 Airborne sampling and optical sensing methods for Macro scale mapping

RRES worked with Burkard to develop the MVI air sampler (see picture below). This device was built at Burkard and was tested in a wind-tunnel facility there. It has been patented and is able to sample air for viable *Sclerotinia* spores, incubate them and make a detection using a biosensor, with the result sent by SMS text along with hourly weather data. Burkard and RRES are continuing to adapt the device for DNA-based detection using (LAMP) assays. LAMP stands for Loop-mediated Isothermal Amplification.

In 2014 several hyperspectral measurements were carried out in two fields of winter wheat. One of the fields was infected with the fungal disease “yellow rust” (*Puccinia striiformis*), while the other field contained healthy wheat. The measurements were carried out with two identical spectrometers (Q-wave – RGB Lasersystems GmbH), with a spectral range from 380 till 950 nm and a resolution of 0.5 nm. One of the spectrometers was used to measure the crop reflection and the other to measure the solar radiation (figure 1).



Figure 1. Spectrometers used. The reference spectrometer measures the solar radiation and the measure spectrometer measures the crop reflection.

During three days in May and June 2014, 336 hyperspectral reflections were sampled from the yellow rust infected field (figure 2) and 307 samples from the healthy field (figure 3).



Figure 2. Yellow rust infection in the experimental field.



Figure 3. Experimental field with unaffected wheat

All data were processed by the statistical classification plugin “PerClass” in Matlab. Reflectance was calculated by dividing the crop reflection by the solar radiation. In order to reduce the amount of noise, a “moving average” filter was applied to this reflectance dataset (Figure 4). Spectral range was therefore reduced from 400 till 925 nm with a resolution of 10 nm.

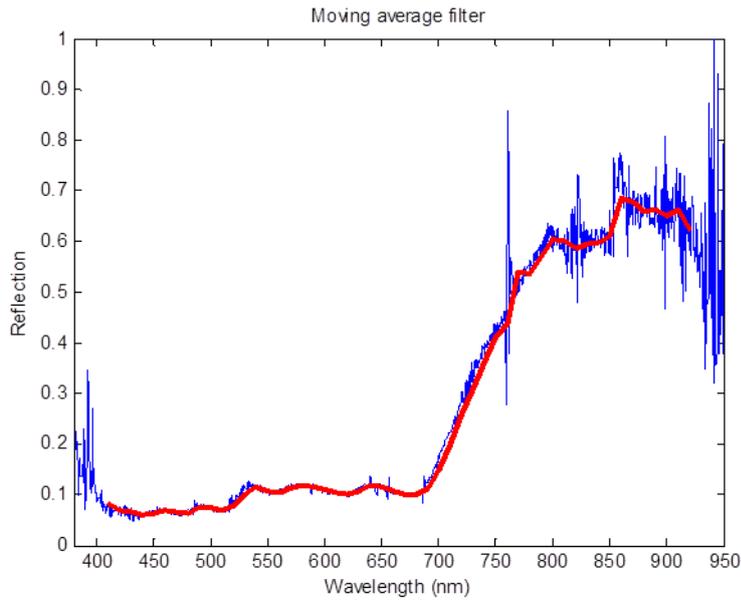


Figure 4. Moving average filter applied to dataset (red), and original data (blue).

The reflectance data was classified with a Fisher Linear Discriminant Analysis (LDA). This type of supervised LDA calculates a function which best separates the class “yellow rust” from the class “healthy wheat”. This function was validated by a “leave-one-out” cross-validation to test the LDA on independent data. The LDA classifier was finally split in individual discriminant weights to determine the most distinctive wavelengths to detect yellow rust in the field.

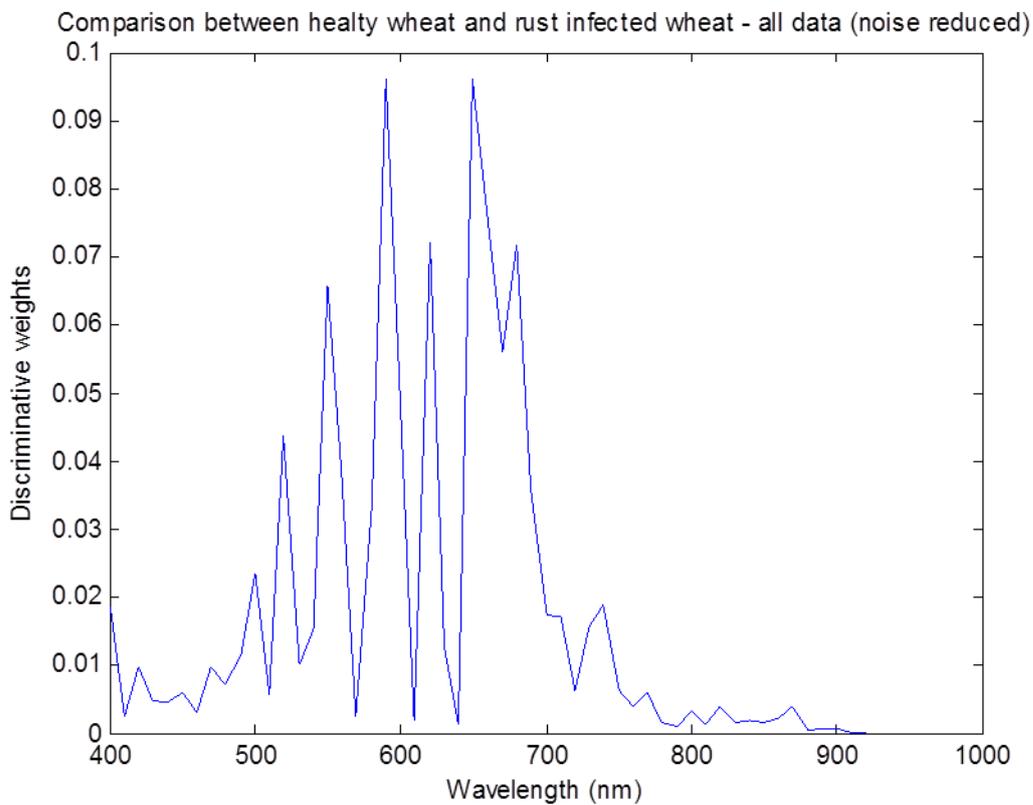


Figure 5. Most distinctive wavelengths to detect yellow rust in winter wheat.

The results from the “leave-one-out” cross-validation show that the Fisher LDA classifier has a classification rate of 99.1% to successfully detect yellow rust. The confusion matrix shows 334 correct classifications from the total set of 336 “yellow rust” measurements. The classification rate of the healthy wheat is 92.8%, corresponding to 291 correct classifications out of 307 in total.

The individual discriminant weights of the LDA shows a selection of 7 most distinctive wavelengths to detect yellow rust in winter wheat: 550, 590, 620, 650, 660, 670 and 680 nm. When reducing the total reflectance data to only those 7 wavelengths, followed by a similar but new Fisher LDA classification on this reduced data set, results in 332 correct classifications of “yellow rust” and 282 correct classifications of “healthy wheat”.

Other scientific publications show similar distinctive wavelengths: Zhang et al. (2014) finds wavelengths at 574, 633 and 689 nm for the detection of yellow rust in winter wheat. Mewes et al. (2011) is capable to detect mildew in wheat with a selection of wavelengths at 495, 526, 557, 587, 725 and 740 nm. Especially the green, red and red-edge spectral bands are similar to our findings.

From a plant-physiological perspective the distinctive wavelength at 550 nm and the ones from 650 till 680 nm, can be explained possibly by the yellow discolorations of the wheat (at 550 nm) due to the fungal infection and a decrease in chlorophyll-a leading to ineffective photosynthesis and a higher reflection in the red band (from 650 till 680 nm). Research from Zhao et al. (2014) shows a significant decrease in chlorophyll-a when wheat is infected by yellow rust.

These findings should be repeated in new field tests to validate the distinctiveness of the selected wavelengths to detect yellow rust in winter wheat, even in a premature phase.

### **3.2 Molecular based diagnostic tools for micro scale identification**

#### **Air sampling**

Air sampling has been coordinated by Rothamsted Research (RRes). It was performed with a Burkard 7 day recording volumetric spore trap at six locations, Wageningen (NL), North Roof (UK), Slagelse (DK), Field Lab (FL); Geescroft (GA) and Little Hoos (LH). Air sampling has been performed in (19-10 / 02-11) October 2011, (20-04 / 30-04) April 2012, (31-05 / 09-06) June 2012, (03-11 / 13-11) November 2012, (03-05 / 12-05) May 2013 and (07-06 / 16-06) June 2013. The spore traps were placed on top of tall buildings in urban surroundings in order to avoid direct influence on sampling from close by farmland (Figure 1, annex 1). The aim is to obtain air samples which are representative of the general fungal species composition in air at a regional scale. A spore trap was also operated at ground level in an experimental field at RRES. Samples from this spore trap have also been included in the first pilot experiment. Based on meteorological data and visual assessments of the tape a representative series of samples were selected for DNA extraction. Samples from autumn 2011 to spring 2013 have been used for optimization and development of final protocols.

#### **DNA extraction**

Samples were sent to RRES, where each daily section (48mm x 20mm of sticky film onto which spores and other airborne particles are impacted) was divided into two sub-samples, each placed into a 2 ml screw-top tube. DNA was extracted from each sub-sample by shaking in a Fastprep machine with microscopic glass beads in an extraction buffer, followed by DNA purification. DNA pellets were resuspended in 30µl of sterile deionised water for use in further DNA analysis. DNA was extracted from a total of 250 samples.

#### **NGS platform**

Samples from October 2011, April 2012, June 2012, November 2012, May 2013 and June 2013 from Denmark, Holland and UK, respectively, have been selected for 454 amplicon sequencing using PCR primers amplifying the ITS1 region (Internal Transcribed Spacer 1) in the ribosomal DNA of fungal species. PCR primers were tagged with DNA barcodes and applied on individual samples for sample identification after pooling and sequencing. Pools of DNA amplicons were sent to Eurofins MWG for sequencing on a GS junior 454 sequencer (Roche Diagnostics). Sequences were quality filtered and clustered into OTUs (Operational Taxonomic Units) at 99% identity and BLAST searches were performed for the most abundant OTUs. Among the most abundant fungal species were several yeast fungi, *Cladosporium* species, *Didymella exitalis* and *Mycosphaerella graminicola*. Among all OTUs the

most abundant plant pathogenic fungi were: *Didymella exitialis*, *Mycosphaerella graminicola*, *Botryotinia fuckeliana*, *Microdochium nivale*, *Ramularia collo-cygni*, *Verticillium dahlia*, *Blumeria graminis*, *Fusarium oxysporum*, *Itersonilia perplexans* (flower/petal blight), *Lewia infectoria* (*Alternaria*), *Epicoccum nigrum*. Based on these NGS results and an inventory of literature a range of fungal species of agricultural importance were selected for targeted detection and quantification using the real-time PCR platform. Due to DNA secondary structure or primer mismatch rust species were not among the identified OTUs. However, it was decided to include these species in the real-time PCR platform as the rust species are producing airborne spores which can migrate over long distances and may cause severe epidemics in cereals (see below for results).

### Real-time PCR platform

An inventory of literature described TaqMan real-time PCRs have been made and when necessary optimized for air sample usage. It was decided to analyse the air samples with the following 17 TaqMan PCRs: Generic fungi, different *Fusarium* species (*F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae*, *F. verticillioides*, *F. langsethiae*, *F. sporotrichioides*), *Microdochium nivale*, *Leptosphaeria maculans*, *Leptosphaeria biglobosa*, *Mycosphaerella graminicola*, *Mycosphaerella graminicola*, *Sclerotinia sclerotiorum*, *Puccinia striiformis*, *Puccinia graminis*, *Puccinia triticina* and *Verticillium dahlia*. Genomic DNA of the different targets have been collected. Primers and TaqMan probes ordered, are shown in table 1 (see Annex1). All (TaqMan) real-time PCRs have been evaluated and when needed optimized for specificity and one annealing temperature. Simultaneous detection and quantification of the different targets can be performed under the same PCR conditions. Figure 2 (see Annex 1) shows the result of the generic fungal amplification of the 240 collected samples and 16 target specific TaqMan PCRs detecting two different corresponding target concentrations in one run (Figure 3, Annex 1).

The 16 fungal species specific TaqMan based PCRs have been used for identification and quantification of 250 total DNA extracted air samples. Figure 4 and 5 in Annex 1) and table 2-18 (see Annex 1) summarize the content of the 250 air samples collected at the six locations, Wageningen (NL), North Roof (UK), Slagelse (DK), Field Lab (FL); Geescroft (GA) and Little Hoos (LH). Air sampling has been performed in (19-10 / 02-11) October 2011, (20-04 / 30-04) April 2012, (31-05 / 09-06) June 2012, (03-11 / 13-11) November 2012, (03-05 / 12-05) May 2013 and (07-06 / 16-06) June 2013. High concentrations of *Puccinia striiformis*, *Puccinia graminis*, *Puccinia triticina* have been identified.

### 3.3 A new generation decision engine for IWM (Integrated Weed Management)

A generic decision support system (DSS) concept was designed and made operational as an online calculation tool on this URL address:

<http://130.226.173.145/cp/weeds/parameters.asp?Language=en&ID=PURE>.

This work was done in close collaboration with Robert Leskovcek and colleagues from KIS (WP3), Arnd Verschwele from JKI (WP3), Nicolas Munier-Jolain from INRA and Roberta Masin and colleagues from CNR.

This DSS prototype integrates the 'best parts' from 3 existing DSS, as identified in the EU project ENDURE (2007-2010). Firstly, 'DecidHerb', supported by INRA, which contribute algorithms to quantify short- and long term needs for weed control. Secondly, 'GestInf', supported by CNR, which contributes to calculation functions to quantify the expected economic net return of alternative weed control options. And thirdly, 'CPOweeds', supported by AU, which contributes to herbicide dose-response functions and calculation functions to optimize compositions of herbicide tank-mixes.

The integrated DSS prototype includes 3 calculation tools:

- 'Problemsolver'. The user supports a field report. The tool quantifies the need for weed control on a weed species level and recommends herbicide treatment options including optimized herbicide 'tank-mixtures', sorted descending for the expected economic net return.
- 'Efficacy Profile'. The user selects a herbicide product and accompanying conditions. This tool calculates the expected efficacy from 4 dose rates relating to the registered dose (N): 2/1N, 1/1N, 1/2N and 1/4N on a weed species level. This tool also calculates dose rates required to meet specific levels of efficacy.

- 'Users mixture'. The user selects a herbicide 'tank-mixture', which may include 2-4 herbicide products and calculates the expected efficacy from the mixture and each mixing component.

**DSS for Integrated Weed Management** Menu and news per 22<sup>th</sup> May 2013

Test version  
Weeds > Problemsolver > Treatment options

Treatment options, sorted by Cost < Go back Print Close

No.	Trade names	Dosage (unit/ha)		Cost Euro/ha	Eco. Net Return (Euro/ha)	Weed species	Efficacy	
		Actual	Normal				Actual	Target
<input type="checkbox"/>								
<input type="checkbox"/> 1	Clio	0,065 l		0,15 l	1.5	Chenopodium album	92%	92%
	Totals				1.5	301		
<input type="checkbox"/> 2	Zeagran ultimate	0,17 l		2 l	3.4	Chenopodium album	92%	92%
	Totals				3.4	299		
<input type="checkbox"/> 3	Mechanical control:	1 pass		1 pass	20.0	Chenopodium album	95%	92%
	Totals				20.0	297		

Harrowing, light: Adjust techniques and conditions to achieve as a minimum, the following results:

- 10 cm from crop row
- 2 cm deep
- 2 cm ridging in row
- 3 hours of dry soil after treatment

As these 3 tools are powered by a joint 'decision engine', full integrity between output from these tools is automatically secured.

The IT-system architecture consists of a generic code developed in the programming languages ASP.net and JAVA, while all agronomic relations including user-interfaces have been designed in integrated in a series of Microsoft SQL Server databases. By editing these databases, operational DSS prototypes and versions suitable for distribution were customized for arbitrary conditions and combinations, e.g. country, crop, weed species, classes of weed growth stage, classes of temperatures, classes of water stress, herbicides and herbicide dose-response functions, herbicide adjuvants, etc.

This DSS concept was customized for 3 countries (Slovenia (Slo), Italy (It), Germany(Ger)), 1 crop (maize), 2 principles for quantification of needs for weed control (reliable/very safe or reliable/risky, previously developed categories that should give the same advise) and 2 levels of expected 'robustness' (in terms of weed control), which resulted in a total of 12 DSS prototypes.

These 12 prototypes include 70 herbicides (Slo: 21, Ita: 20, Ger: 29), 92 weed species (Slo: 16, Ita: 15, Ger: 61), 4 classes of weed growth stage and 6.769 parametrized herbicide dose-response functions. Some herbicides and weed species are the same in different countries. The required datasets were collected and converted into parameters in the DSS in time, before the season of weed control in maize in the 3 countries in the spring of 2013 and 2014.

Based on output from WP3, which showed that the efficacy of herbicides registered for pre-emergence (pre-em) use, was not significantly different from early post-emergence application of the same herbicides, the DSS prototypes systematically replaced pre-em herbicide applications with early post-emergence applications. This change strongly supports several IPM principles.

As mechanical control options could not be executed in the planned field validation trials in WP3, only a few mechanical scenarios were integrated, and only to demonstrate the functional integrity. This may be examined in recommendation for treatment no. 3 and 7 in this prototype: <http://130.226.173.145/cp/weeds/Parameters.asp?CropID=3140&UnderSownID=3140&CultivarID=0&YieldID=2&GrowthStageID=16&SoiltypeID=5&SoilmoistID=3&SoilstructureID=2&TMinID=8&TMaxID=14&WaterStressID=1&SeasonID=1&Optimize=1&RotationIDList=&SpecialProblemIDList=&WeedID=3080&WeedGSID=2&WeedCountID=3&WeedID=-1&WeedGSID=-1&WeedCountID=-1&Language=en&ID=PURE>.

The DSS also integrates relevant legal restrictions on the use of herbicides, which are also subjected to EU-cross compliance (which may affect EU-subsidies for farmers).

In close collaboration with WP3, a total of 12 field validation trials were planned (3 countries x 2 years x 2 farms) and executed in maize in Slovenia, Italy and Germany in 2013 and 2014. Unfortunately, due to massive drought (Slovenia) and massive rain (Germany) 2 out of 12 field validation trials were lost. In order to support publication, KIS and JKI will repeat trials (with their own funds) in 2015.

The results indicate that one or more DSS prototypes have performed (in terms of weed control and yield) at equal level with local 'best practice' reference treatments, and some DSS prototypes also demonstrated strong potentials for reducing the input of herbicides without jeopardizing crop safety:

- Slo: DSS reduced Treatment Frequency Index by 20-40%
- Ita: DSS reduced cost by +/- 10-20%
- Ger: DSS reduced Treatment Frequency Index by +60 - -70% (under varying standards).

However, more data and work is required to include more crops, weeds, herbicides (and alternative control options) and 'conditions' required by professional farmers. The DSS possesses generic qualities in terms of 1) biological models behind the DSS and 2) IT system architecture, which is considered to be suitable and potent for upscaling in additional crops and countries.

The results from field validation also indicate that the DSS design allows for identification of weeds species, weed growth stages and weed density classes, before decisions on control are made. This strongly support principles of Directive 2009/128/EC, Annex 3. In total, this DSS design specifically complies with 6 (and soon 7) of the 8 general principles listed there.

No	Principle	DSS
1	Crop rotation, fertilization, soil cultivation, etc.	No
2	Monitoring	Yes
3	Thresholds	Yes
4	Non-chemical control	Yes
5	Targeted use of herbicides	Yes
6	Use of herbicides on necessary levels	Yes
7	Anti-resistance strategies	Yes
8	Evaluation	Yes

### 3.4 Mating disruption solutions for pest control in protected vegetables and grapevine

The development of a new method of mating disruption is warranted because numerous insect pests, most notably leafhoppers and planthoppers (*Hemiptera*) do not rely on long-range chemical communication. In these insects mate recognition and localization of the partner are mediated exclusively via substrate-borne vibrational signals and their populations are currently managed primarily by insecticide treatments. Surprisingly, although males use special species-specific disruptive vibrational signals to interfere with the courtship of rivals, mating interruption by induced vibrations has been rarely considered even from a theoretical viewpoint and there has been virtually no research on how to exploit this common insect communication channel as a tool for pest control. Here we present the development, evaluation and improvement of a mating disruption system based on substrate-borne vibrations. The present document describes additional experiments performed to evaluate and improve the effectiveness of vibrational disruption for *Scaphoideus titanus* control in field and under laboratory conditions, as well as further analysis of the 2013 field data.

#### 1. Temporal activation – night time.

Field experiments were conducted from July to September 2014 using the latest prototype (2nd version) of the device from 2013.

Pairs of insects were released in net sleeves including one vine shoot (8-12 leaves) each in a test vineyard. The plants, on which net sleeves were suspended, were vibrated by the shaker using one of six temporal activation variants programmed with the control panel (Figure 6). The aim was to determine periods when it can be turned off without losing any efficacy in controlling the insect mating, focusing on the night period. Three rows of the vineyard were simultaneously managed with the method and the trials were randomized to minimize the effect of season.

The protocol was the following: one pair of insects from the rearing was put in each of 10 cages in the evening (7 pm) and collected after 13 hours; then after one week, females were dissected to determine whether mating occurred or not.

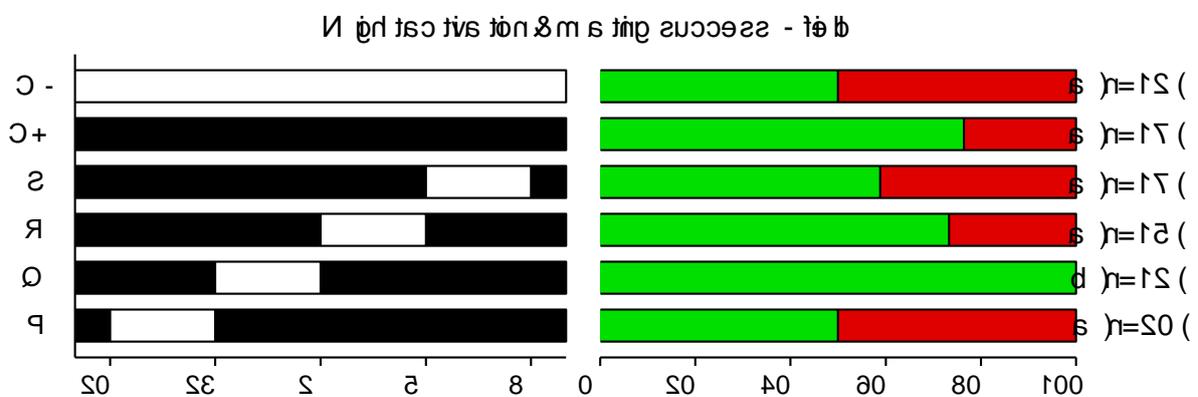


Figure 6. Temporal activation experiment. % of virgin and mated females after any treatment (periods off on - black area/off - white area/on; virgin females – green/mated females – red). Different letters indicate significant difference after Chi squared followed by Ryan’s test for multiple comparisons. n = number of replications for each treatment.

The only period that stood out was “Q” (disruption inactive between 11pm and 2am) with zero mating success, although the sample size was rather low because of problems with rearing and weather during the 2014 experimental season. Therefore, we cannot talk about differences between different periods of inactivation during the night with certainty.

#### 2. Temporal activation – additional analysis

To understand the mechanism of the diel pattern of *S. titanus* activity, we obtained weather data for the 2013 experimental season from the meteorological station of San Michele all’Adige and compared

it with the summarized result of field trials in the same season – namely, switching off mini-shakers between 10 AM and 6 PM does not decrease efficiency of disruption, meaning that the insects are inactive during that time.

Averaging the environmental parameters throughout the season, the daily pattern could be compared to insect activity. Average hourly temperature had a roughly sinusoidal pattern (Figure 7), with minimum at 6 AM and maximum at 4 PM, and humidity the exact opposite. Wind speed at 10 m above the ground had three daily peaks, at 2 AM, 11 AM and 6 PM, of those the 1800 hours peak was by far the most prominent, with the value reaching 4.4 m/s. Combined silent windows of trials that did not result in diminished efficiency of disruption (between 10 AM and 6 PM) roughly coincided with the warmest part of the day, although the period of mean hourly temperature above 22°C, as was at 10 AM, extended well into the evening after the insect mating resumed again. The silent windows covered almost all the range of wind speeds, but matched exactly the peak of light intensity. We can conclude that light intensity entrains insect activity, not temperature, humidity or wind speed.

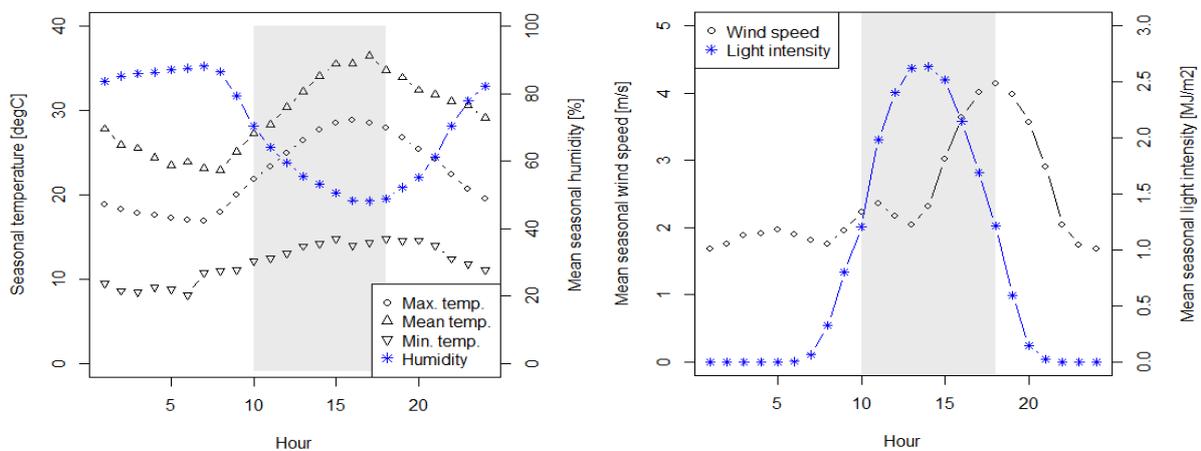


Figure 7. Comparison between averaged environmental parameters and the period of insect inactivity (shaded). Left: temperature and humidity; right: wind speed and light intensity.

### 3. Measuring attenuation in the field

The shaker hung on the wire produced 7.5 mm/s peak amplitude, measured on its body in the axis of movement, and the amplitude and frequency spectrum changed slightly with transmission to the wire (see Figure 9 for experimental set up).

At week 0 (before the growing season), the amplitude of vibrations measured on the wire varied non-monotonously with distance, probably representing the pattern of reflections from poles. The signal at the reference point 4 cm away from the source had 8.2 mm/s peak amplitude, and dropped to 0.13 mm/s at the last measuring point 40 m away (Figure 8). After the plants attached, however, the attenuation was more pronounced, with amplitude dropping to the 0.01 mm/s range less than 20 m away from the source. Non-monotonous amplitude variation with distance was not observed anymore. The frequency of the strongest peak varied from 140 to 275 Hz at different locations and times, but was between 190 and 200 Hz in most cases.

Extra attenuation was observed at the wire-plant interface. Within a few centimeters' distance, amplitude fell almost 23 dB, from 0.72 mm/s on the top wire to 0.05 mm/s on the stem next to it. With transmission down the stem, peak amplitude remained in the same range, and still reached 0.04 mm/s at the bottom of the shoot. The amplitude on the leaves was higher, reaching 0.19 mm/s on the upper leaf and 0.13 mm/s on the bottom leaf, the latter representing approximately 15 dB attenuation relative to the wire.

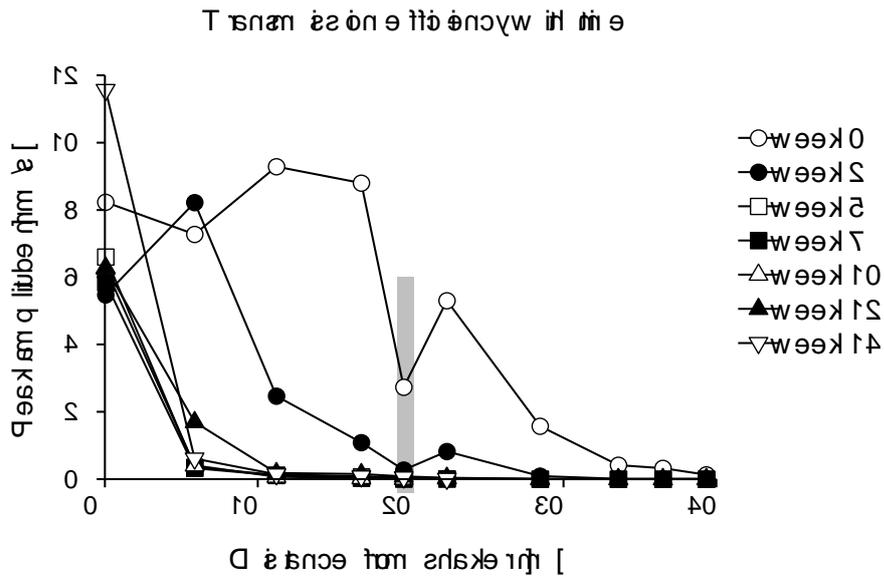


Figure 8. Peak amplitude of the disruption signal recorded at different points along the supporting wire in different parts of the season (2013). Grey band denotes the recording point near a pole, other points were positioned approximately in the middle of each wire section delimited by poles.

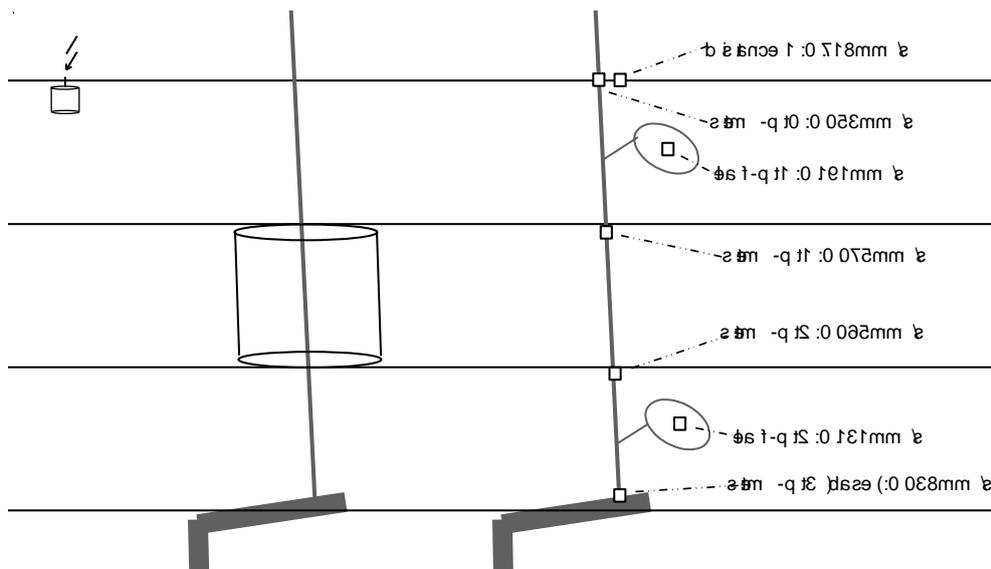


Figure 9. Diagram of cage placement for animal trials (left) and the locations for measuring transmission of vibrations down the grapevine shoot. A mini-shaker was affixed to the upper wire (draw in the upper left corner).

Combining this information with the measured threshold for efficiency of disruption in laboratory conditions (15  $\mu\text{m/s}$ ), the effective range of the prototype mini-shaker is believed to be 10 meters along the wire to either side of the device.

#### 4. Effect of duty cycle – laboratory trials

The objective of this experiment was to examine whether disruption is also efficient if it is activated in an on-off pattern on a short time scale. For example, 1:1 pattern would mean 50% energy saving, 1:2 pattern 66.7% energy saving, etc.

Arena consisted of a grapevine cutting with two leaves, male placed on the bottom leaf and female on the top leaf. Playback was delivered via a mini-shaker attached to the stem below the bottom leaf petiole, and noise signal was artificially generated from white noise, band-filtered under 150 and

above 350 Hz. Amplitude was set above the absolute threshold determined by »threshold for disturbance effect« trials, to approximately 18  $\mu\text{m/s}$  peak, measured at the top leaf.

Three duty cycles were tested: 1 on and 1 off, 1 on and 1.5 off, 1 on and 2 off, with 0.5s as a unit. So, 1:1 means 0.5s on and 0.5s off, 1:1.5 means 0.5s on and 0.75 s off, 1:2 means 0.5s on and 1s off. The animals were allowed to start signaling, and disruption was triggered when the male started to search, then left for 20 minutes. After 20 min (or less, if the male came into contact with female), the outcome was noted as one of three options:

- \* failure: male didn't progress further than the stalk of his starting leaf, only CD+LoD emission.
  - \* same leaf/courting: male arrived to the top leaf with the female, to within a few cm or opposite leaf lamina, and/or started courting (CrD). This option would likely succeed in mating given more time.
  - \* direct contact: male touched the female while courting, would almost surely succeed in mating.
- Every duty cycle was tested with 20 replications. Pattern had significant effect on success. The 1:1 pattern was 100% efficient in preventing mate localization, while the other two had significantly lower efficiency, comparing the "failure" results (Figure 10).

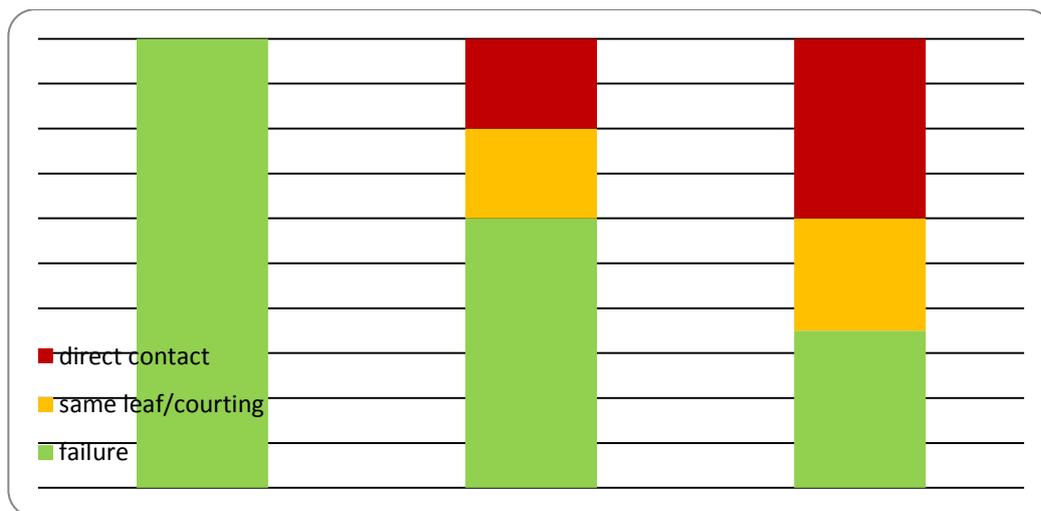


Figure 10 Searching success with different duty cycles.

Looking at recordings, we found that 0.5 s period of silence is too short to accommodate both a male pulse and a female reply, preventing mate recognition, but any longer period of silence enables at least some males to locate the female and proceed with courtship. From this, we conclude that only the duty cycle of 0.5s on and 0.5 off would provide maximum energy saving while still efficient for mating disruption.

##### 5. Effect of vibration on choice of substrate – laboratory trials (preliminary).

The objective of this experiment was to examine whether vibrational noise affects the animals' choice of substrate (perching/feeding). A trial consisted of releasing 10 females inside a transparent plexiglass cube with four grapevine cuttings, two of which were vibrated, and noting their position for 23 hours.

Cuttings were almost as tall as the cube's side, positioned each in own corner not touching the walls or each other, and had comparable leaf area. They were connected at the top with metal binders to a pair of metal wires running cleanly through holes on two sides of the cube, parallel to side walls. Two wires, each connected to two plants (Figure 11). Remaining hole area was plugged gently with cotton to prevent animals escaping while allowing the wires to vibrate freely. Outside, a B&K mini-shaker, attached to one wire with wax, played a constant disturbance stimulus. The shaker could be disconnected and reattached to the other wire as needed, so either one or the other pair of plants was vibrated at the same time (randomized to exclude the effect of plants and box sides). Amplitude of vibrations was checked prior to each trial at measuring points in the middle of topmost and bottommost leaf of each plant. Playback amplitude was set so the vibration reached at least 15  $\mu\text{m/s}$  peak amplitude on all points of the vibrated pair. Non-vibrated pair was also checked for air-transmitted vibration amplitude.

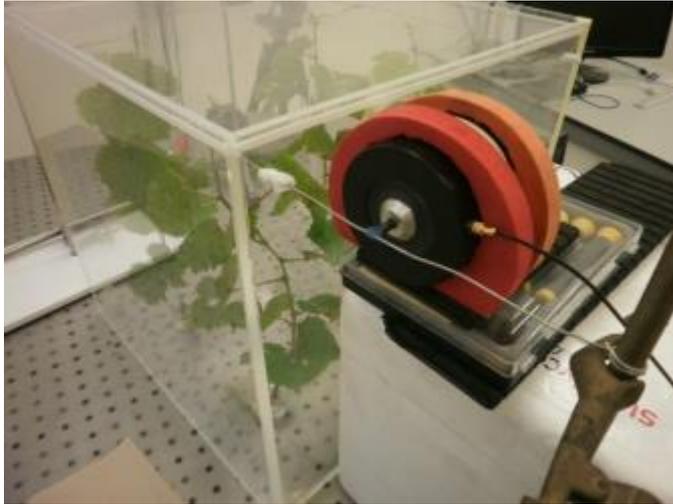


Fig 11: experimental setup for the choice of substrate trials.

A trial began at 9 AM by placing a small plastic pot with 10 animals inside in the middle of the cube's floor, approx. same distance to all the plants and opening the cover, then closing the cube's "ceiling". The animals were counted after 3, 6, 12 and 23 hours from outside, determining the number on each plant and walls/wires/pot (additional category was dead or missing animals). After the 12h count (9 PM), the light in the lab was turned off and turned on again before the 23h count at 8 AM the following day to simulate day/night cycle.

Only the results after one full day are presented; this was the only period where more than half of females released were consistently found on the plants (as opposed to walls, wires or the starting pot), and percentages are less reliable with lower numbers. In 4 out of 10 trials, the distribution is exactly 50/50, and females did not clearly favour either side in other trials (Figure 12), so average percentage of females standing on vibrated plants is not different from 50.

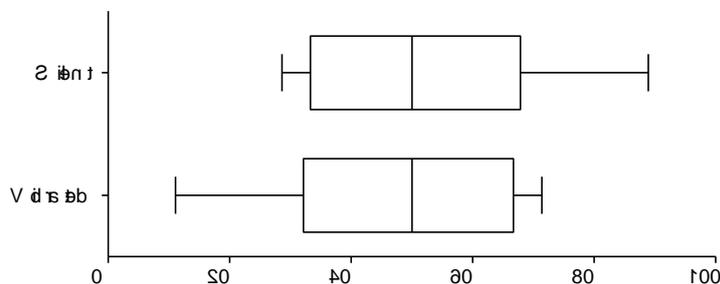


Figure 12. Percentage of females standing on non-vibrated ("silent") and vibrated plants after 10 trials.

From these results, we cannot conclude that *S. titanus* females are either attracted or repelled by strong vibratory noise present continuously for longer periods of time in their habitat. Unfortunately, only females could be tested with sufficient replications because of problems with rearing. Repeating this experiment with males could potentially yield more interesting results, as sexually-active males perform "call & fly" behaviour (females are more sedentary), and there were slight indications from other field and lab experiments in these two years that males treat disturbance noise as a cue that something is going on.

## 6. Effect of vibration on mating duration – laboratory trials (preliminary).

Does noise influence mating duration? Stress due to perceived presence of a rival male could cause a copulating *S. titanus* male to shorten or prolong mating. Additionally, males sometimes emit vibrational signals (more specifically the "buzz" element) during copulation; the function of this is unknown, but it could be affected by vibratory disturbance noise, thereby possibly influencing copulation itself. If copulation was shortened, this could negatively impact sperm transfer, meaning lower reproductive success and an additional effect of artificial disruption on populations of *S. titanus* in the field.

We tested this hypothesis using pairs of *S. titanus* placed on a same grapevine leaf and allowed to court and mate. When the male emitted a calling signal, received a reply from the female and started to search, continuous playback of disruption noise was started using a mini-shaker connected to the section of stem below the leaf. Amplitude of this disturbance was set at 5  $\mu\text{m/s}$  measured in the middle of the leaf, which is below the amplitude that prevents communication, but well within the natural range. After courting was complete, the animals were left to copulate until they broke the contact by themselves, all the time with disruption noise playback on. Six preliminary trials were conducted, yielding average time of copulation  $4121 \pm 276$  s, compared with 3750 s in the control trial. Duration of actual copulation was never shorter than one hour. On average, duration of copulation in noisy conditions is slightly longer, although not in all cases. Statistical comparison would not be meaningful because of low number of replications. Therefore, we cannot conclude that vibrational disruption would negatively influence copulation in *S. titanus*.

### 7. Study on the potential to apply the method to alternative pests (whiteflies).

The aim of the study was a detailed description of vibrational communication of *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) with the view to its potential exploitation in pest management by disruption of reproductive behaviour. To accomplish the aims we established a rearing from which we took individuals for trials in bioacoustic lab. In some experiments individuals (either males or females) were surgically deprived of wings to allow a direct observation of the body during signal emissions.

**Rearing.** The insects were reared in the greenhouse on potted tobacco (*Nicotiana tabacum*) and bean (*Phaseolus vulgaris*) plants. To obtain virgin individuals, tobacco leaves with puparia were removed and isolated in containers. After adults emerged, they were sexed under a stereomicroscope and males and females were then kept on cut bean leaves in separate containers.

**Recording.** Insects were placed in a recording arena which consisted essentially of a cut bean leaf in a plastic container. Substrate-borne vibrations were recorded from the bean leaf by a laser vibrometer. Recording of substrate-borne vibrations was in some cases accompanied also by video camera recording (Figure 13).

Experiments performed: intact individuals, no video: a couple (a male and a female), one female and two males, multiple females and males.

Data are currently processed for analysis. In general, whiteflies have a relatively wide repertoire of vibrational signals which are used by both males and females during courtship, before copula. It is thinkable to test external vibrations as interference to abate the rate of matings within the population.

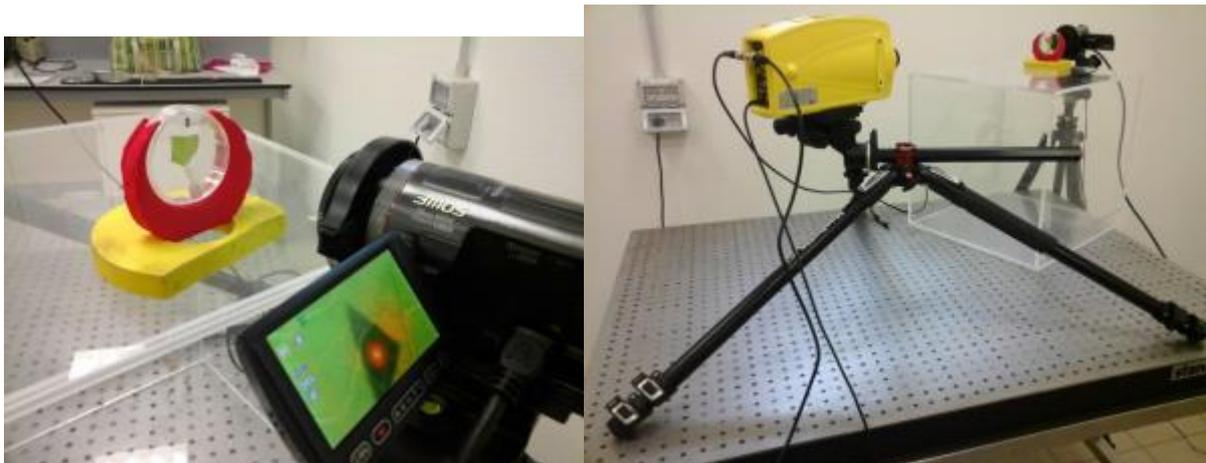


Figure 13. Photos of the set-up of recording of the mating behaviour and associated vibrational signals. Insects were included in small petri dishes (diameter 5 cm) and placed on an anti-vibrational table where they were simultaneously video and audio (laser vibrometer) recorded.

Following on the results of 2013 in beginning 2014 (i.e. performed by CNR and DLO) slow release dispensers with *Tuta absoluta* pheromone have been manufactured and sent to partner CNR to perform field experiments in tomato crops in Italy. Samples of dispensers were sent back to the Netherlands regularly to check the release rate. Treatments were: pheromone treatment + biocontrol; pheromone treatment + chemical control compared with standard chemical and biological control

treatments. Trials were performed in the spring-summer cycle and in the summer-autumn cycle. Planning was to also send the biodegradable dispensers, but the manufacturer was not able to produce them, so the original dispensers with comparable release rate were used. The field experiments have been performed by CNR and results have been reported at the PURE-meeting in January 2015.

Slow release dispensers with *Tuta absoluta* pheromone have been used by CNR to perform false trail following experiments in tomato in Italy. Also several types of biodegradable dispensers were set out in the field experiments to compare the evaporation rate of the pheromone from these dispensers with the standard ones. Samples of dispensers have been sent regularly to DLO to estimate the release rate. Plant damage and insect countings were performed by CNR.

### **3.5 Canopy density sensing based crop protection in vegetable and orchards**

#### **Spray deposition measurements**

In 2014 the prototype of the precision orchard sprayer (Canopy Density Spraying, CDS) is for a second year tested in a commercial orchard. During the season applied spray volume of the CDS-sprayer was measured with and without the use of sensors. Reductions in applied spray volume of 20%-50% could be achieved due to the adaptation of spray volume based on measured tree canopy structure with the sensors.

Spray deposition measurement were carried out to quantify spray distribution in tree canopy in a commercial apple and pear orchard (July). Spray deposition of the applied spray volumes was measured in the tree segmented in a top, center and bottom section of the tree. Spray distribution of the CDS- sprayer is compared with a standard cross-flow fan sprayer and the precision sprayer without the use of sensors (KWH) with two nozzle types; standard hollow cone nozzles (ATR) and drift-reducing venturi nozzle types (TVI).

#### **Spray deposition in pear trees**

Applied spray volume in the V-shape pruned pear trees was for the conventional cross-flow fan sprayer 150 l/ha, for the KWH precision orchard sprayer equipped with ATR hollow cone nozzles 165 l/ha and with TVI venturi type nozzles 180 l/ha. Maximum spray volume for the CDS sprayer using ATR hollow cone nozzles was 290 l/ha and for the venturi nozzles 300 l/ha. Spray deposition for the five spray techniques is presented in Figure 14 as measured in  $\mu\text{l}/\text{cm}^2$  leaf area in the top, center and bottom part of the pear tree. Average spray deposition over the three tree sections is for the standard cross-flow fan sprayer  $0.47 \mu\text{l}/\text{cm}^2$ , for the KWH sprayer equipped with ATR nozzles  $0.39 \mu\text{l}/\text{cm}^2$  and with TVI nozzles  $0.51 \mu\text{l}/\text{cm}^2$ , showing that average spray deposition of the KWH sprayer in the tree is similar as of the standard cross-flow fan sprayer when using the TVI nozzles and lower when using the ATR nozzles. When the sensor is used to detect crop canopy and nozzles switch on/off when crop canopy is detected it is shown that average spray deposition in the pear trees for the CDS sprayer both with the ATR nozzles ( $0.13 \mu\text{l}/\text{cm}^2$ ) as with venturi TVI nozzles ( $0.35 \mu\text{l}/\text{cm}^2$ ) is lower than of the standard sprayer and of the KWH sprayer used without the sensor.

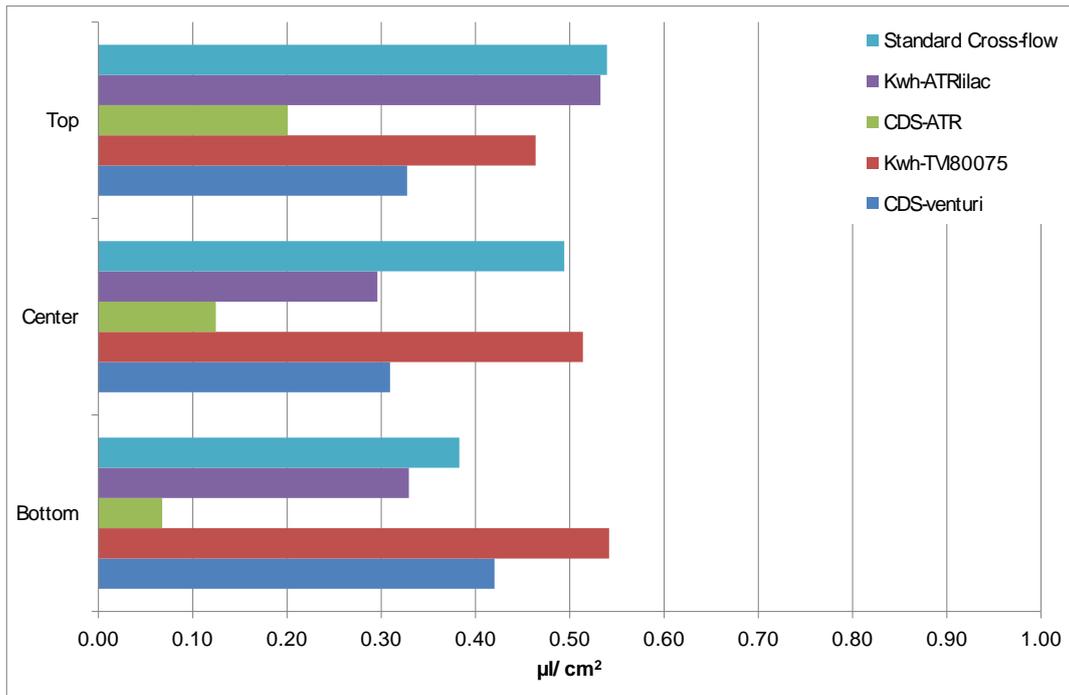


Figure 14. Spray deposition ( $\mu\text{l}/\text{cm}^2$ ) in V-shape pear trees for a standard cross-flow fan sprayer, a KWH cross-flow fan sprayer with two nozzle types and a CDS sprayer with two nozzle types adapting spray volume to tree canopy.

Applied spray volume was for the CDS sprayer with ATR nozzles 135 l/ha and with the venturi nozzles 130 l/ha resulting in spray volume reductions of resp. 18% and 29% compared to the KWH cross-flow fan sprayer with similar nozzle types. For the CDS sprayer with venturi nozzle types spray deposition was only in the bottom part of the V-shape pear trees comparable to the deposition of the standard cross-flow fan sprayer. Both in the center and the top section of the tree spray deposition was lower. Spray deposition of the CDS sprayer with ATR hollow cone nozzles was in all three tree sections much lower than of the standard cross-flow fan sprayer. Variation in spray deposition over tree sections is for the CDS sprayer and the KWH sprayer both using venturi type nozzles comparable or less (better; CV resp. 17% and 8%) than of the standard cross-flow fan sprayer (CV 17%). However spray deposition on individual leaves within tree sections show for all spray techniques large variations (CVs 30%-70%).

### Spray deposition in apple trees

Applied spray volume in the spindle dwarf apple trees was for the conventional cross-flow fan sprayer 165 l/ha, for the KWH precision orchard sprayer equipped with ATR hollow cone nozzles 180 l/ha and with TVI venturi type nozzles 195 l/ha. Maximum spray volume for the CDS sprayer using ATR hollow cone nozzles was 315 l/ha and for the venturi nozzles 330 l/ha. Spray deposition for the five spray techniques is presented in Figure 15 as measured in  $\mu\text{l}/\text{cm}^2$  leaf area in the top, center and bottom part of the apple tree. Average spray deposition over the three tree sections is for the standard cross-flow fan sprayer  $0.59 \mu\text{l}/\text{cm}^2$ , for the KWH sprayer equipped with ATR nozzles  $0.50 \mu\text{l}/\text{cm}^2$  and with TVI nozzles  $0.71 \mu\text{l}/\text{cm}^2$ , showing that average spray deposition of the KWH sprayer in the apple tree is higher or similar as of the standard cross-flow fan sprayer when using resp. the TVI nozzles or the ATR nozzles. When the sensor is used to detect crop canopy and nozzles switch on/off when crop canopy is detected it is shown that average spray deposition in the apple trees for the CDS sprayer both with the ATR nozzles ( $0.33 \mu\text{l}/\text{cm}^2$ ) as with venturi TVI nozzles ( $0.45 \mu\text{l}/\text{cm}^2$ ) is lower than of the standard sprayer and of the KWH sprayer used without the sensor.

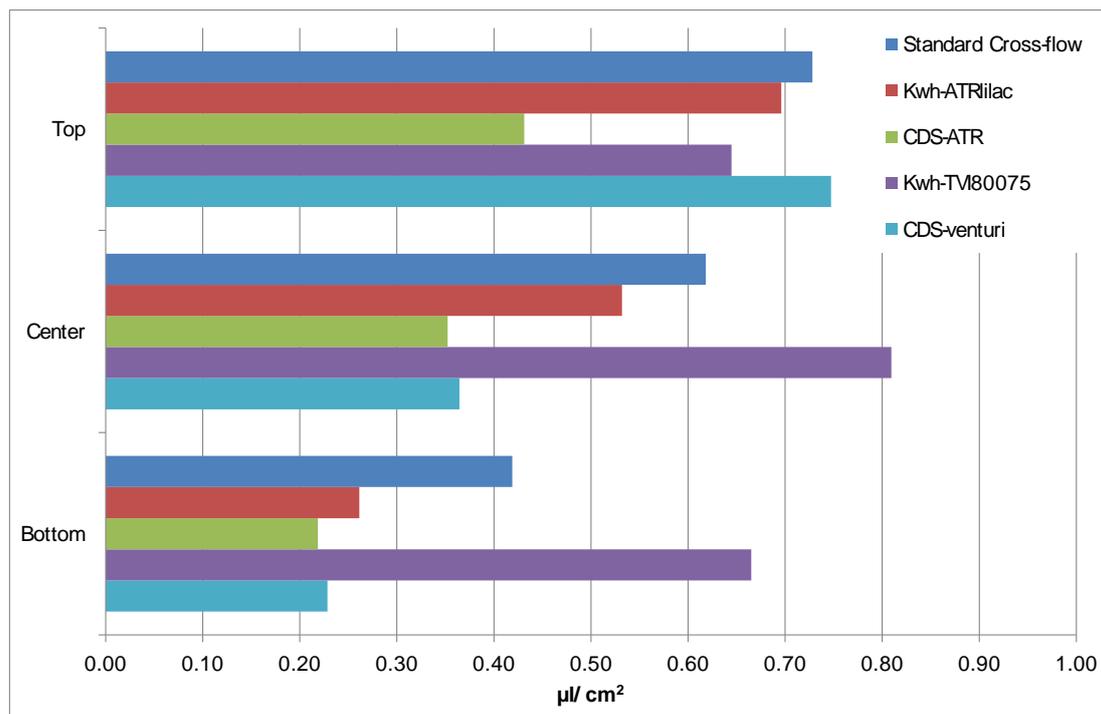


Figure 15. Spray deposition ( $\mu\text{l}/\text{cm}^2$ ) in spindle dwarf apple trees for a standard cross-flow fan sprayer, a KWH cross-flow fan sprayer with two nozzle types and a CDS sprayer with two nozzle types adapting spray volume to tree canopy.

Applied spray volume was for the CDS sprayer with ATR nozzles 165 l/ha and with the venturi nozzles 170 l/ha resulting in spray volume reductions of resp. 7% and 14% compared to the KWH cross-flow fan sprayer with similar nozzle types. For the CDS sprayer with venturi nozzle types spray deposition was only in the top part of the spindle dwarf apple trees comparable to the deposition of the standard cross-flow fan sprayer. Both in the center and the bottom section of the tree spray deposition was lower. Spray deposition of the CDS sprayer with ATR hollow cone nozzles was in all three apple tree sections much lower than of the standard cross-flow fan sprayer.

## 4 Conclusions

### 4.1 Airborne sampling and optical sensing methods for Macro scale mapping

We conclude that it is feasible to use reflectance measurements to identify yellow rust infected wheat. The identification of wavelengths that are distinctive to detect yellow rust in winter wheat with reflectance measurements was possible at both DLO and RRES. However, the correct identification of the disease severity was still rather low. Therefore, we do not recommend to use this technique to quantify the disease severity with reflectance measurements at this moment. Further work is needed to enhance the specificity of the technique and capability to detect early infection stages.

The MVI air sampler was built and tested in a wind-tunnel. It has been patented and is able to sample air for viable *Sclerotinia* spores, incubate them and make a detection using a biosensor, with the result sent by SMS text along with hourly weather data. The device is being adapted for DNA-based detection using LAMP assays.

### 4.2 Molecular based diagnostic tools for micro scale identification

For six air-sampling periods it was possible to obtain fungal material at six different locations. DNA was successfully extracted and used in two different platforms for molecular detection and quantification of fungal pathogens. A generic method using 454 amplicon sequencing of the ITS region revealed a baseline of relatively few highly abundant fungal species such as several yeast fungi, *Cladosporium* sp. and *Didymella* sp. Several plant pathogenic fungi were also detected. Specific simultaneous detection and quantification of 16 different targets with TaqMan could be performed under universal PCR conditions on a 384 real-time machine. Real-time TaqMan can be used as a

molecular based diagnostic tool for micro scale identification and monitoring of air borne fungal pathogens.

#### **4.3 Decision engine for IWM**

The results indicate that one or more DSS prototypes have performed (in terms of weed control and yield) at equal level with local 'best practice' reference treatments, and some DSS prototypes also demonstrated strong potentials for reducing the input of herbicides without jeopardizing crop safety:

- Slovenia: DSS reduced Treatment Frequency Index by 20-40%
- Italy: DSS reduced cost by +/- 10-20%
- Germany: DSS reduced Treatment Frequency Index by +60 - -70% (under varying standards).

However, more data and work is required to include more crops, weeds, herbicides (and alternative control options) and 'conditions' required by professional farmers. The DSS possess generic qualities in terms of 1) biological models behind the DSS and 2) IT system architecture, which is considered to be suitable and potent for upscaling in additional crops and countries.

The results from field validation also indicate that the DSS design allows for identification of weeds species, weed growth stages and weed density classes, before decisions on control are made. This strongly support principles of Directive 2009/128/EC, Annex 3. In total, this DSS design specially complies with 6 (and soon 7) of the 8 general principles listed there.

#### **4.4 Mating disruption solutions for pest control in protected vegetables and grapevine**

In order to evaluate and improve the effectiveness of vibrational disruption for *Scaphoideus titanus* control, additional experiments under field and laboratory conditions were conducted in 2014, as well as an extended analysis of the 2013 field data. The aim was to determine periods when the device can be turned off without losing any efficacy in controlling the insect mating, and optimal distances between the devices along the wire. The following conclusions could be drawn:

- Light intensity strongly influences the insect activity, not temperature, humidity or wind speed and suggests that the device may be switched off during 11 pm and 2 am. Although the field experimental data seem to confirm this conclusion, the low number of available insects in the 2014 trial does not validate a statistical valid conclusion.
- The effective range of the prototype mini-shaker is believed to be 10 meters along the wire to either side of the device.
- A duty cycle of 0.5s on and 0.5 off provides maximum energy saving and sufficient mating disruption.

Slow release dispensers with *Tuta absoluta* pheromone have been manufactured and field experiments in tomato crops in Italy were performed. Trials were performed in the spring-summer cycle and in the summer-autumn cycle and show good results.

#### **4.5 Canopy density sensing based crop protection in vegetable and orchards**

The prototype CDS sprayer has been constructed and tested in commercial fruit orchards. The sprayer has a laser scanner to measure canopy density, and with that sensor and appropriate algorithms developed canopy density spraying has been made possible. The field trials of 2014 show that the target speed of 7.5 km/h is currently not yet feasible to perform well in apple and pear trees. After the end of the PURE project the CDS sprayer will be developed further and focus will be on increasing the driving speed.

## 5 Annex I



Figure 1. Spore trap placed at rooftop level in Slagelse, Denmark



PURE – Deliverable D11.8

Target name	Target position	Forward primer	Reverse Primer	TaqMan Probe
Consensus Fungus	5.8S	AAC TTT CAACAACGGATCTCTTGG	GCGTTCAAAGACTCGATGATTCAC	6-FAM CATCGATGAAGAACGCAGCGAAATGC <b>BHQ1</b>
<i>Sclerotinia sclerotiorum</i>	calmodulin gene	CCCAGTTTCGACTCTCCTCTTTTAT	AACTCAGACTCGGAAGGGTTTGT	6-FAM AGACATCTTGACCGACCCGCCCC <b>BHQ1</b>
<i>Puccinia striiformis</i>	ITS1	TGAACCTGCAGAAGGATCATT	TGAGAGCCTAGAGATCCATTGTTA	6-FAM TAAGACTTG/ZEN/GITGCATGATTTGAAAGAATCATI <b>IABkFQ</b>
<i>Puccinia graminis</i>	ITS1	TGAACCTGCAGAAGGATCATT	TGAGAGCCTAGAGATCCATTGTTA	6-FAM TTGTGGCTC/ZEN/GACTCTCTTATAAACCAAACC <b>IABkFQ</b>
<i>Puccinia triticina</i>	ITS1	TGAACCTGCAGAAGGATCATT	TGAGAGCCTAGAGATCCATTGTTA	6-FAM TGAAGAAT/ZEN/CATTGTGATTAAGTATACGTGGCAITCT <b>IABkFQ</b>
<i>Fusarium graminearum</i>	Ammonium Ligase	CGTACGTCTCACTTCAAGCCAG	GGACACCTTGGTCATCCATAGAG	6-FAM CACCACATCAACTCCT <b>MGBNFQ</b>
<i>Fusarium culmorum</i>	SCAR	TCACCCAAGACGGGAATGA	GAACGCTGCCCTCAAGCTT	6-FAM CACTTGGATATATITCC <b>MGBNFQ</b>
<i>Fusarium avenaceum</i>	SCAR	CCATCGCCGTGGCTTTC	CAAGCCCACAGACACGTTGT	6-FAM ACGCAATTGACTATTGC <b>MGBNFQ</b>
<i>Fusarium poae</i>	SCAR	AAATCGGCGTATAGGGTTGAGATA	GCTCACACAGAGTAACCGAAACCT	6-FAM CCCAACCGACCCTI <b>MGBNFQ</b>
<i>Microdochium nivale</i>	SCAR	CGCCAAGGACTCCTCCAGTAG	GCCGACGAATGGATATTAAGAACT	6-FAM TCCCGCCTTACGGTGGAAAAGC <b>BHQ1</b>
<i>Fusarium verticillioides</i>	Fum1	ATGCAAGAGGCGAGGCAA	GGCTCTCAGAGCTTGGCAT	6-FAM CAATGCCATCTTCTTG <b>MGBNFQ</b>
<i>Fusarium langsethiae</i>	SCAR	GTTGGCGTCTCACTTATTATTC	TGACATTGTTTCCAGATAGTAGTCC	6-FAM cacaccCataCctaCgtgtaa <b>BHQ1 (Capitel=LNA)</b>
<i>Fusarium sporotrichioides</i>	SCAR	GGTTGGCGTCTCACTTATAC	AATTTCTGATTCGCTAAAGTGG	6-FAM ccacaccCAtagTtacGtgtaa <b>BHQ1 (Capitel=LNA)</b>
<i>Leptosphaeria maculans</i>	Actine	cgcgcaggaaaacagattttt	gaagctggaattgagttagcatgtac	6-FAM cgtgcttctgccgctctagcg <b>BHQ1</b>
<i>Leptosphaeria biglobosa</i>	Actine	cgcacTcgaaatgtCcatt ( <b>Capitel=LNA</b> )	tcgcaggccacgtcag	6-FAM taacTcTgTtCcagcttccattg <b>BHQ1 (Capitel=LNA)</b>
<i>Mycosphaerella graminicola</i>	B-tubulin	GCCTTCCTACCCACCATGT	CCTGAATCGGCATCGTTA	6-FAM TTACGCCAAGACATTC <b>MGBNFQ</b>
<i>Verticillium dahliae</i>	ITS	CCGGTCCATCAGTCTCTCTG	ACTCCGATGCGAGCTGTAAC	6-FAM cggGtcCgcCactgc <b>BHQ1 (Capitel=LNA)</b>

Table 1. List of primers and probes used in TaqMan PCR for detection of 16 different fungal species.

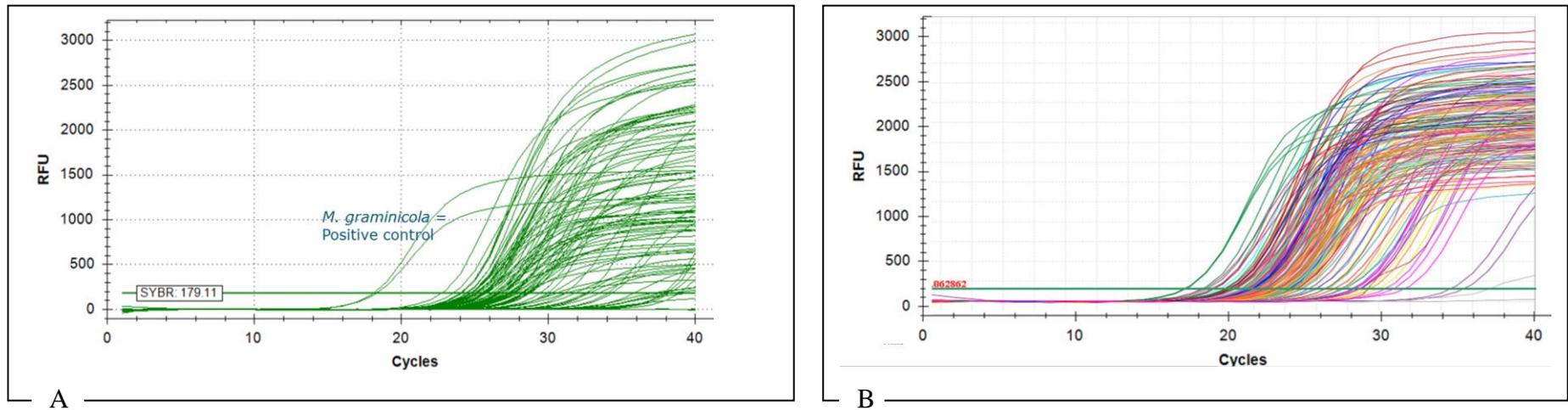


Figure 2. A: DNA air samples collected in Oct 2011 and Apr 2012 and tested with All Fungi TaqMan PCR; B: DNA air samples collected in June & Nov 2012 and May & June 2013 and tested with All Fungi TaqMan PCR. RFU is the relative fluorescence unit. A high RFU level indicates detection of fungal DNA in the air sample. The number of cycles required to get a fluorescence reading is an indication of the amount of fungal DNA present in the sample.

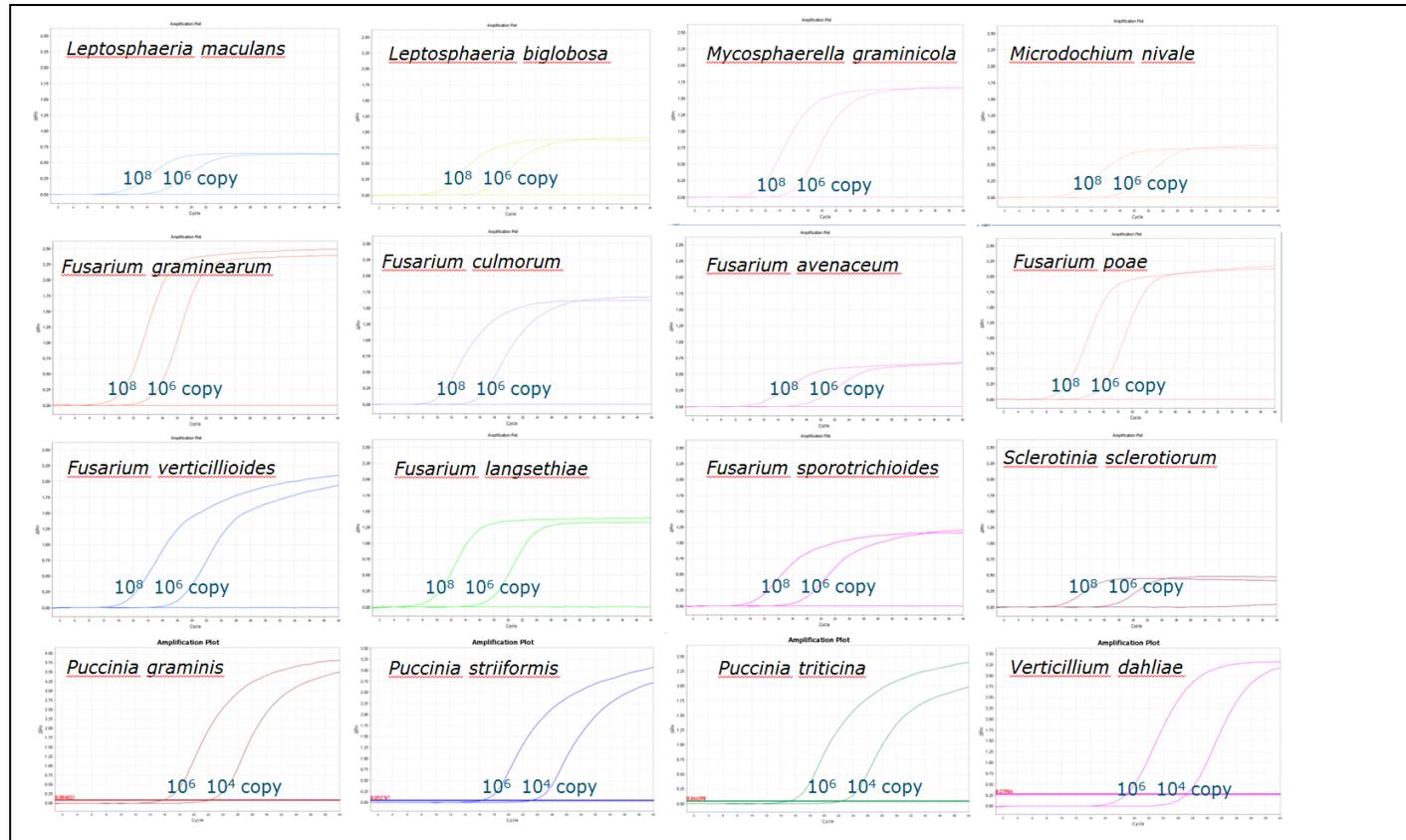


Figure 3. Evaluation of 16 target specific TaqMan PCRs for two different corresponding target concentrations in one real-time run. RFU is the relative fluorescence unit and plotted on the y axis. A high RFU level indicates detection of fungal DNA in the air sample. The number of cycles (x axis) required to get a fluorescence reading is an indication of the amount of fungal DNA present in the sample.



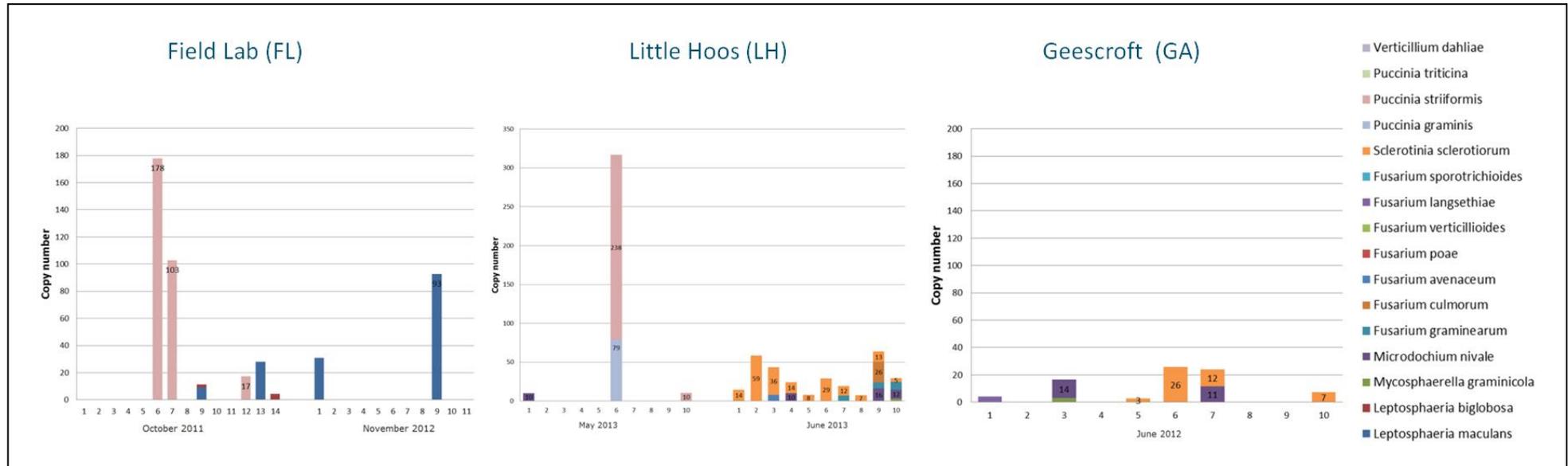


Figure 5. Summary of tested air samples collected at three locations, Field Lab (FL); Geescroft (GA) and Little Hoos (LH), for 16 different fungi. Copy numbers are given for the 16 fungal per time point. Air sampling has been performed in (19-10 / 02-11) October 2011, (20-04 / 30-04) April 2012, (31-05 / 09-06) June 2012, (03-11 / 13-11) November 2012, (03-05 / 12-05) May 2013 and (07-06 / 16-06) June 2013. The higher the copy number, the larger the amount of DNA of that specific species in that specific sample was.

PURE – Deliverable D11.8

	1	2	3	4	5	6	7	8	9	10	11	13	14	15	16	17	18	19	20	21	22	23	
A	Sample copy number	11DK07	11DK15	11NL07	11NL15	NR11	NR19	FL41	12DK04	12DK12	12NL09	RN15	12DK29	12DK37	12NL35	12NR55	12GA74	12DK52	12DK60	12NL57	12NRA13	12NRA21	12FL18
B	Sample copy number	11DK08	11DK16	11NL08	11NL16	NR12	NR20	FL42	12DK05	12DK13	12NL10	*RN15	12DK30	12DK38	12NL36	12NR56	12GA75	12DK53	12DK61	12NL58	12NRA14	12FL11	12FL19
C	Sample copy number	11DK09	11DK17	11NL09	11NL17	NR13	FL35	FL43	12DK06	12NL03	12NL11	RN17	12DK31	12NL29	12NL37	12NR57	12GA76	12DK54	12DK62	12NL59	12NRA15	12FL12	12FL20
D	Sample copy number	11DK10	11DK18	11NL10	11NL18	NR14	FL36	FL44	12DK07	12NL04	12NL12	RN18	12DK32	12NL30	12NL38	12NR58	12GA77	12DK55	12NL52	12NL60	12NRA16	12FL13	12FL21
E	Sample copy number	11DK11	11DK19	11NL11	NR07	NR15	FL37	FL45	12DK08	12NL05	RN11	RN19	12DK33	12NL31	12NR51	12NR59	12GA78	12DK56	12NL53	12NL61	12NRA17	12FL14	MQ
F	Sample copy number	11DK12	11DK20	11NL12	NR08	NR16	FL38	FL46	12DK09	12NL06	RN12	RN20	12DK34	12NL32	12NR52	12NR60	12GA79	12DK57	12NL54	12NL62	12NRA18	12FL15	MQ
G	Sample copy number	11DK13	11NL05	11NL13	NR09	NR17	FL39	FL47	12DK10	12NL07	RN13	MQ	12DK35	12NL33	12NR53	12GA72	12GA80	12DK58	12NL55	12NRA11	12NRA19	12FL16	MQ
H	Sample copy number	11DK14	11NL06	11NL14	NR10	NR18	FL40	FL48	12DK11	12NL08	RN14	MQ	12DK36	12NL34	12NR54	12GA73	12GA81	12DK59	12NL56	12NRA12	12NRA20	12FL17	MQ
I	Sample copy number	13DK11	13DK19	13NL17	13NR15	13LH11	13DK25	13DK33	13NL32	13NR50	13LH46		ATCC18704	ATCC18704	ATCC18704								
J	Sample copy number	13DK12	13DK20	13NL18	13NR16	13LH12	13DK26	13DK34	13NL33	13NR51	13LH47		2000000	2000000	2000000								
K	Sample copy number	13DK13	13NL11	13NL19	13NR17	13LH13	13DK27	13NL26	13NL34	13NR52	13LH48		200000	200000	200000								
L	Sample copy number	13DK14	13NL12	13NL20	13NR18	13LH14	13DK28	13NL27	13NL35	13NR53	13LH49		20000	20000	20000								
M	Sample copy number	13DK15	13NL13	13NR11	13NR19	13LH15	13DK29	13NL28	13NR46	13NR54	13LH50		2000	2000	2000								
N	Sample copy number	13DK16	13NL14	13NR12	13NR20	13LH16	13DK30	13NL29	13NR47	13NR55	13LH51		200	200	200								
O	Sample copy number	13DK17	13NL15	13NR13	13LH9	13LH17	13DK31	13NL30	13NR48	13LH44	13LH52		20	20	20								
P	Sample copy number	13DK18	13NL16	13NR14	13LH10	13LH18	13DK32	13NL31	13NR49	13LH45	13LH53		2	2	2								
													0.2	0.2	0.2								

	19-10 / 02-11-11	20-04 / 30-04-12	31-05 / 09-06-12	03-11 / 13-11-12	03-05 / 12-05-13	07-06 / 16-06-13
Slagelse DK						
Wageningen Radix NL	DK 14	DK 10	DK 10	DK 11	DK 10	DK 10
North Roof UK	NL 14	NL 10	NL 10	NL 11	NL 10	NL 10
Field Lab UK	NR 14	RN 10	NR 10	NRA 11	NR 10	NR 10
Geescroft UK	FL 14			FL 11		
Little Hoos UK					LH 10	LH 10
			GA 10			
	56	30	40	44	40	40

Table 3. Analysis and quantification of the 240 air samples for *Verticillium dahlia* at different locations. The number of copies is given in the table: empty cells indicate no *Verticillium dahlia*, numbers indicate the amount of copies of the target DNA in the sample.

	1	2	3	4	5	6	7	8	9	10	11	13	14	15	16	17	18	19	20	21	22	23	
A	Sample copy number	11DK07	11DK15	11NL07	11NL15	NR 11	NR 19	FL41	12DK04	12DK12	12NL09	RN15	12DK29	12DK37	12NL35	12NR55	12GA74	12DK52	12DK60	12NL57	12NRA13	12NRA21	12FL18
B	Sample copy number	11DK08	11DK16	11NL08	11NL16	NR 12	NR20	FL42	12DK05 70.5	12DK13	12NL10	RN15	12DK30	12DK38	12NL36	12NR56	12GA75	12DK53	12DK61	12NL58	12NRA14	12FL11	12FL19
C	Sample copy number	11DK09	11DK17	11NL09	11NL17	NR 13	FL35	FL43	12DK06	12NL03	12NL11	RN17	12DK31	12NL29	12NL37	12NR57	12GA76	12DK54	12DK62	12NL59	12NRA15	12FL12	12FL20
D	Sample copy number	11DK10	11DK18	11NL10	11NL18	NR 14	FL36	FL44	12DK07	12NL04	12NL12	RN18	12DK32	12NL30	12NL38	12NR58	12GA77	12DK55	12NL52	12NL60	12NRA16	12FL13	12FL21
E	Sample copy number	11DK11	11DK19	11NL11	NR07	NR 15	FL37	FL45	12DK08	12NL05	RN11	RN19	12DK33	12NL31	12NR51	12NR59	12GA78	12DK56	12NL53	12NL61	12NRA17	12FL14	MQ
F	Sample copy number	11DK12	11DK20	11NL12	NR08	NR 16	FL38	FL46	12DK09	12NL06	RN12	RN20	12DK34	12NL32	12NR52	12NR60	12GA79	12DK57	12NL54	12NL62	12NRA18	12FL15	MQ
G	Sample copy number	11DK13	11NL05	11NL13	NR09	NR 17	FL39	FL47	12DK10	12NL07	RN13	MQ	12DK35	12NL33	12NR53	12GA72	12GA80	12DK58	12NL55	12NRA11	12NRA19	12FL16	MQ
H	Sample copy number	11DK14	11NL06	11NL14	NR 10	NR 18	FL40	FL48	12DK11	12NL08	RN14	MQ	12DK36	12NL34	12NR54	12GA73	12GA81	12DK59	12NL56	12NRA12	12NRA20	12FL17	MQ
I	Sample copy number	13DK11	13DK19	13NL17	13NR15	13LH11	13DK25	13DK33	13NL32	13NR50	13LH46		DKS7/100 200000	DKS7/100 200000	DKS7/100 200000								
J	Sample copy number	13DK12	13DK20	13NL18	13NR16	13LH12	13DK26	13DK34	13NL33	13NR51	13LH47		DKS7/100 200000	DKS7/100 200000	DKS7/100 200000								
K	Sample copy number	13DK13	13NL11	13NL19	13NR17	13LH13	13DK27	13NL26	13NL34	13NR52	13LH48		DKS7/100 20000	DKS7/100 20000	DKS7/100 20000								
L	Sample copy number	13DK14	13NL12	13NL20	13NR18	13LH14	13DK28	13NL27	13NL35	13NR53	13LH49		DKS7/100 2000	DKS7/100 2000	DKS7/100 2000								
M	Sample copy number	13DK15	13NL13	13NR11	13NR19	13LH15	13DK29	13NL28	13NR46	13NR54	13LH50		DKS7/100 200	DKS7/100 200	DKS7/100 200								
N	Sample copy number	13DK16	13NL14	13NR12	13NR20	13LH16	13DK30	13NL29	13NR47	13NR55	13LH51		DKS7/100 20	DKS7/100 20	DKS7/100 20								
O	Sample copy number	13DK17	13NL15	13NR13	13LH9	13LH17	13DK31	13NL30	13NR48	13LH44	13LH52		DKS7/100 2	DKS7/100 2	DKS7/100 2								
P	Sample copy number	13DK18	13NL16	13NR14	13LH10	13LH18	13DK32	13NL31	13NR49	13LH45	13LH53		DKS7/100 0.2	DKS7/100 0.2	DKS7/100 0.2								

Slagelse DK	19-10 / 02-11-11	20-04 / 30-04-12	31-05 / 09-06-12	03-11 / 13-11-12	03-05 / 12-05-13	07-06 / 16-06-13
Wageningen Radix NL	DK 14	DK 10	DK 10	DK 11	DK 10	DK 10
North Roof UK	NL 14	NL 10	NL 10	NL 11	NL 10	NL 10
Field Lab UK	NR 14	RN 10	NR 10	NRA 11	NR 10	NR 10
Geescroft UK	FL 14			FL 11		
Little Hoos UK			GA 10		LH 10	LH 10
	56	30	40	44	40	40

Table 4. Analysis and quantification of the 240 air samples for *Puccinia tritici* at different locations. The number of copies is given in the table: empty cells indicate no *Puccinia tritici*, numbers indicate the amount of copies of the target DNA in the sample.

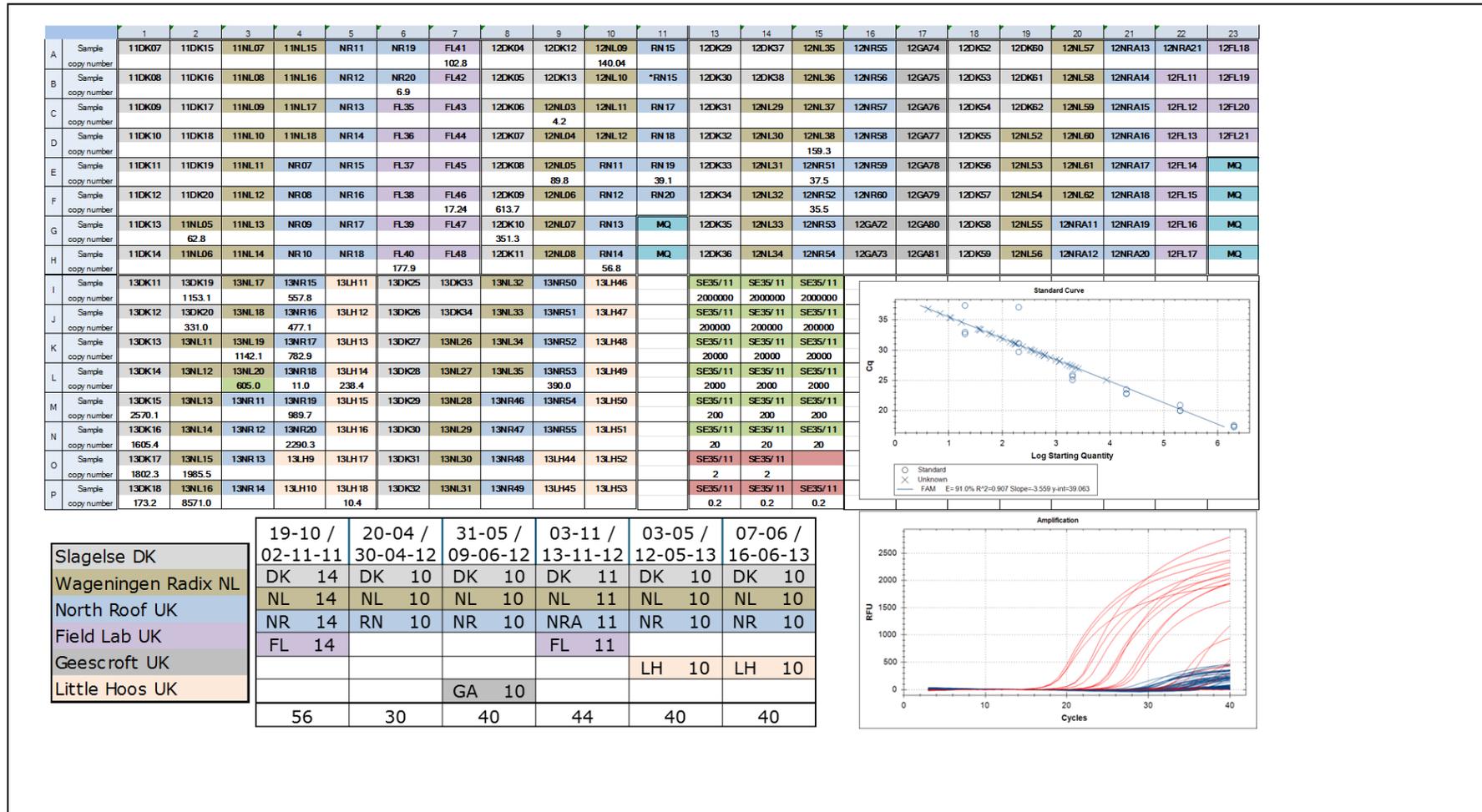


Table 5. Analysis and quantification of the 240 air samples for *Puccinia striiformis* at different locations. The number of copies is given in the table: empty cells indicate no *Puccinia striiformis*, numbers indicate the amount of copies of the target DNA in the sample.

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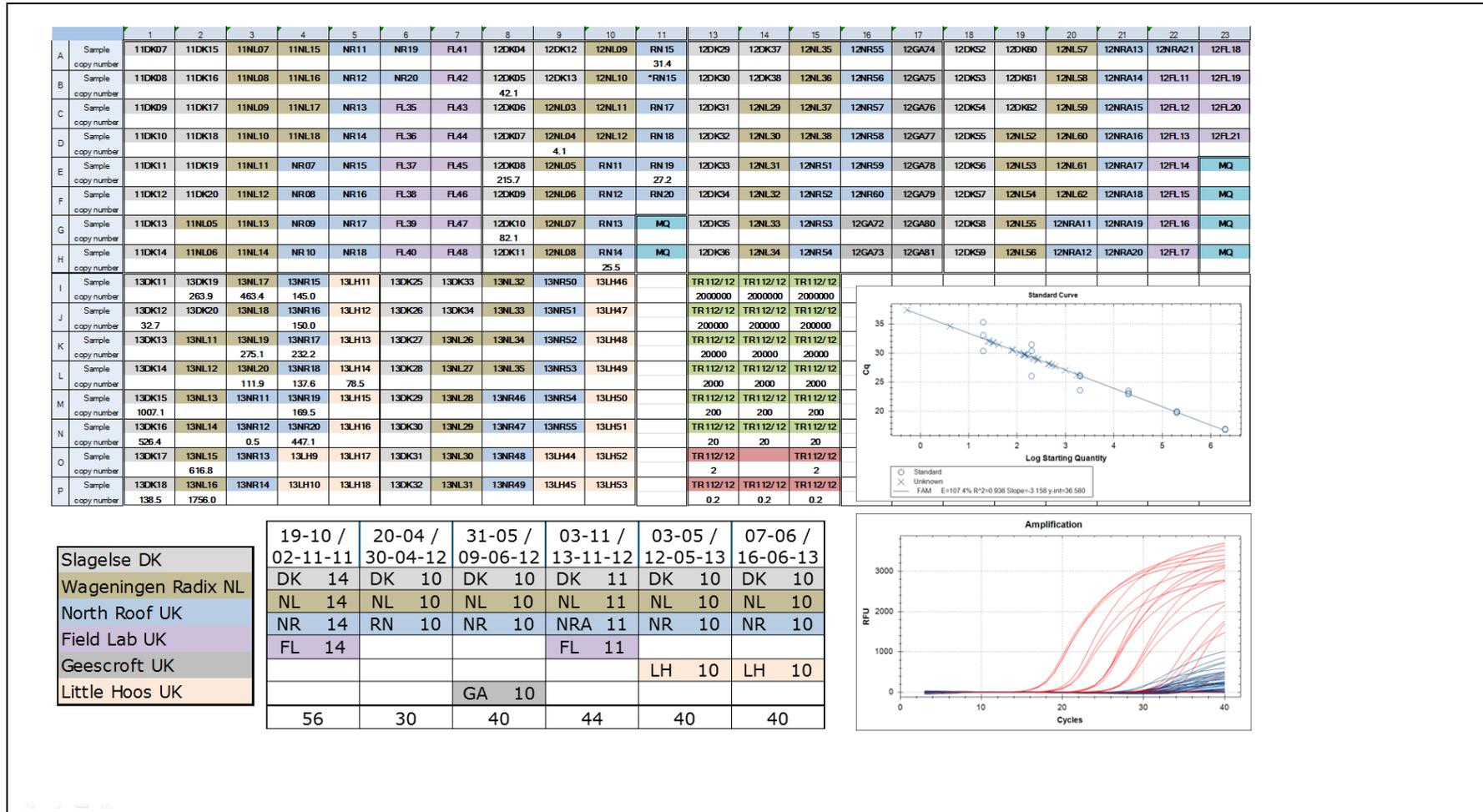


Table 6. Analysis and quantification of the 240 air samples for *Puccinia graminis* at different locations. The number of copies is given in the table: empty cells indicate no *Puccinia graminis*, numbers indicate the amount of copies of the target DNA in the sample.

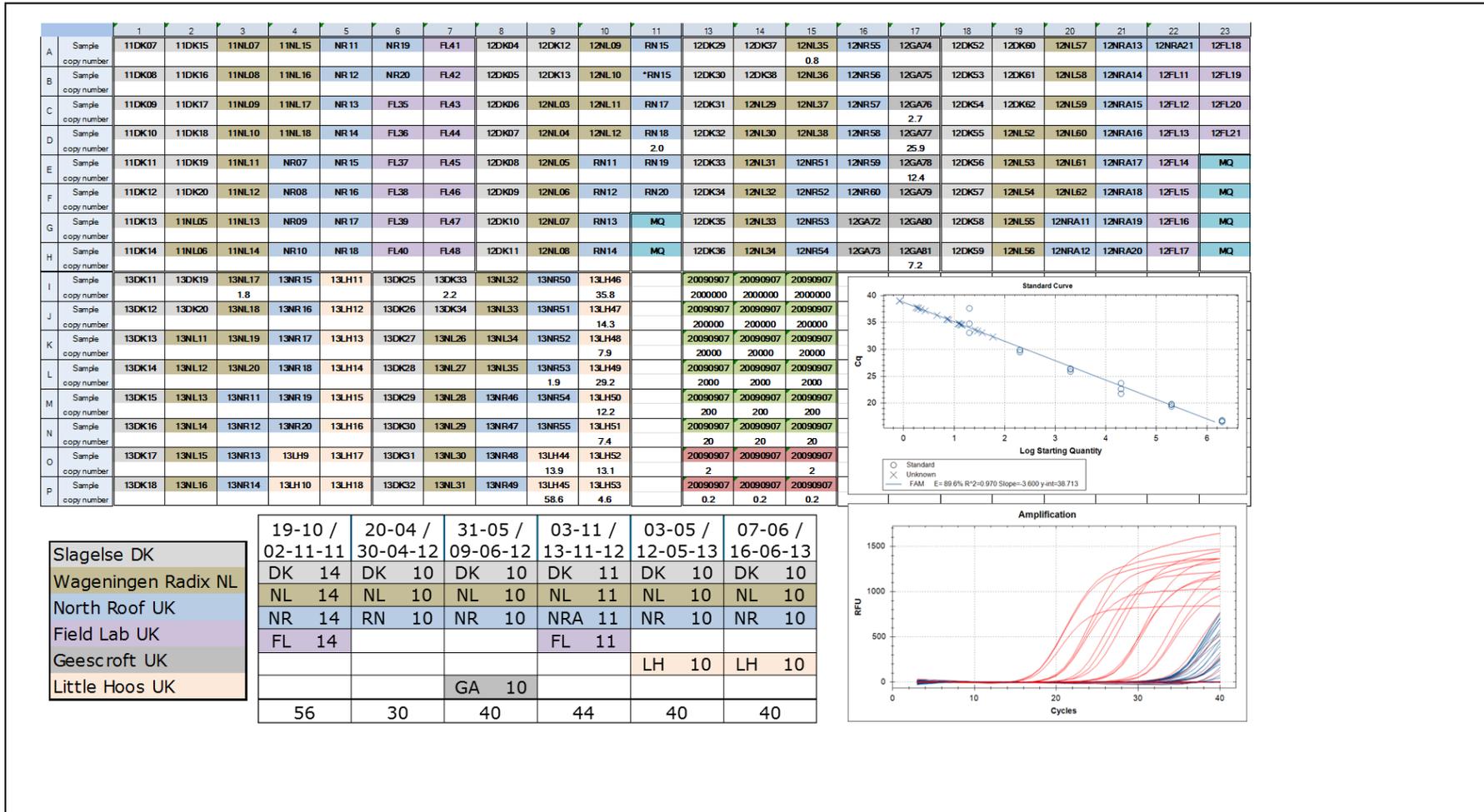


Table 7. Analysis and quantification of the 240 air samples for *Sclerotinia sclerotiorum* at different locations. The number of copies is given in the table: empty cells indicate no *Sclerotinia sclerotiorum*, numbers indicate the amount of copies of the target DNA in the sample.

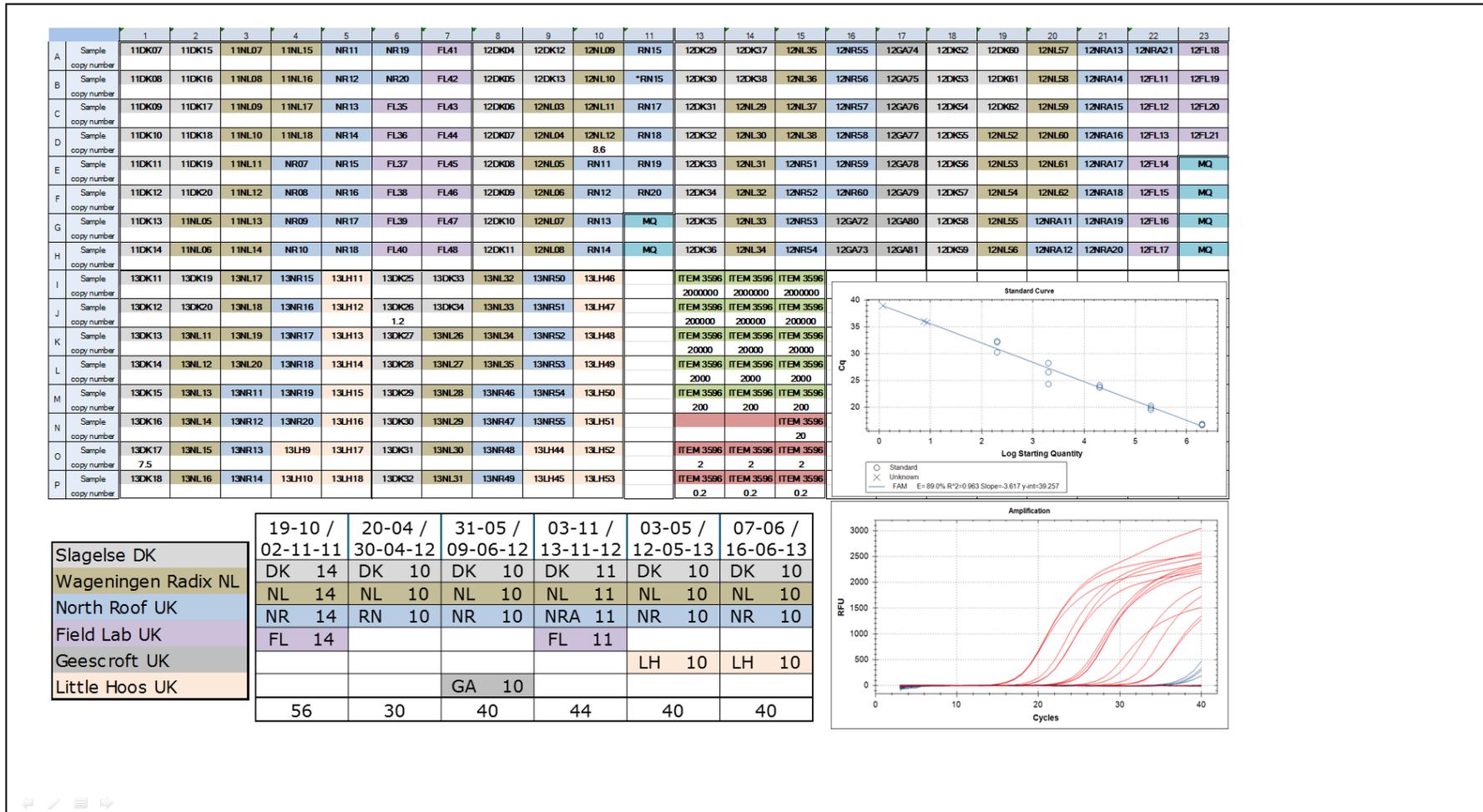


Table 8. Analysis and quantification of the 240 air samples for *Fusarium sporotrichioides* at different locations. The number of copies is given in the table: empty cells indicate no *Fusarium sporotrichioides*, numbers indicate the amount of copies of the target DNA in the sample.

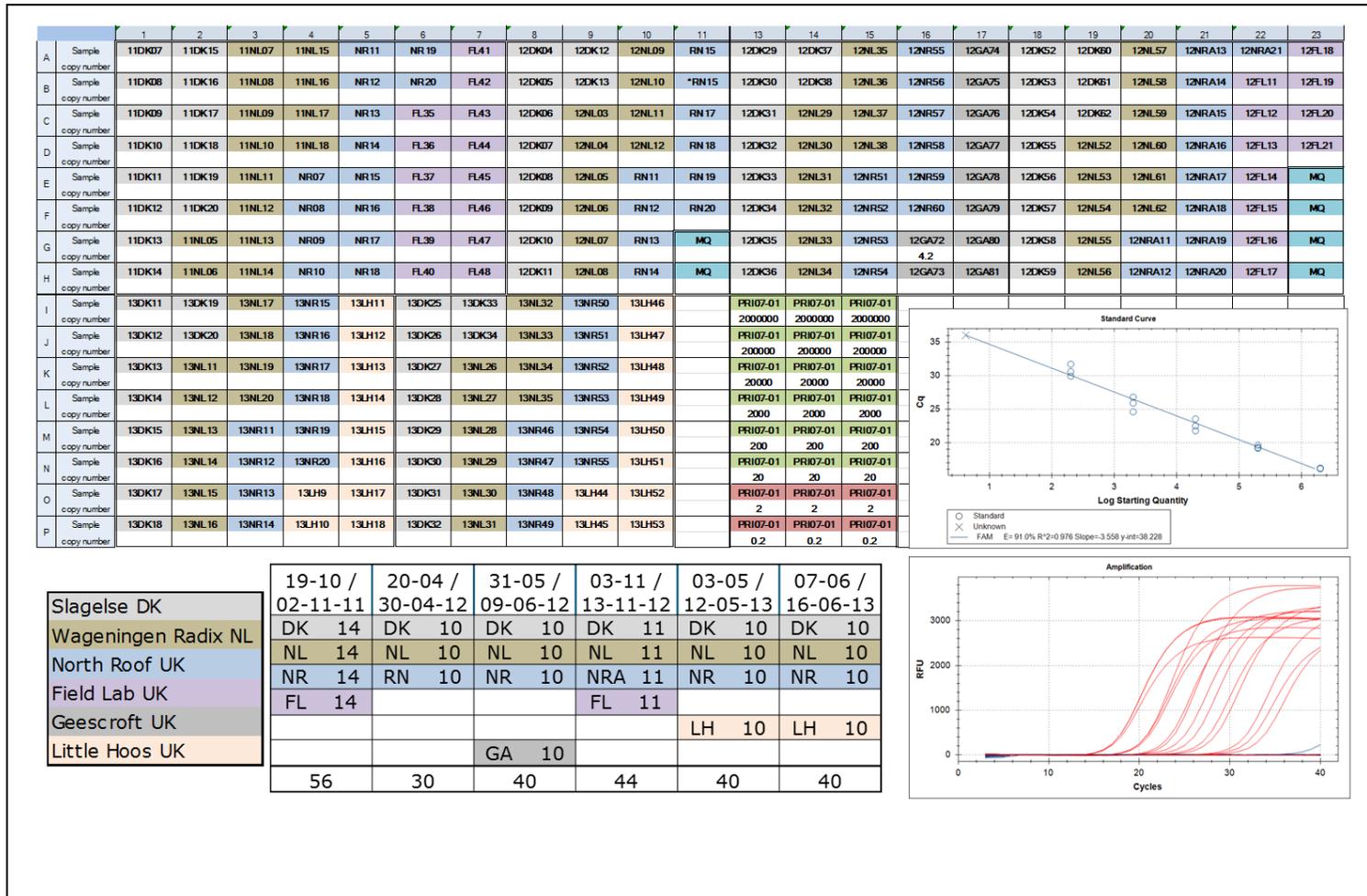


Table 9. Analysis and quantification of the 240 air samples for *Fusarium langsethiae* at different locations. The number of copies is given in the table: empty cells indicate no *Fusarium langsethiae*, numbers indicate the amount of copies of the target DNA in the sample.

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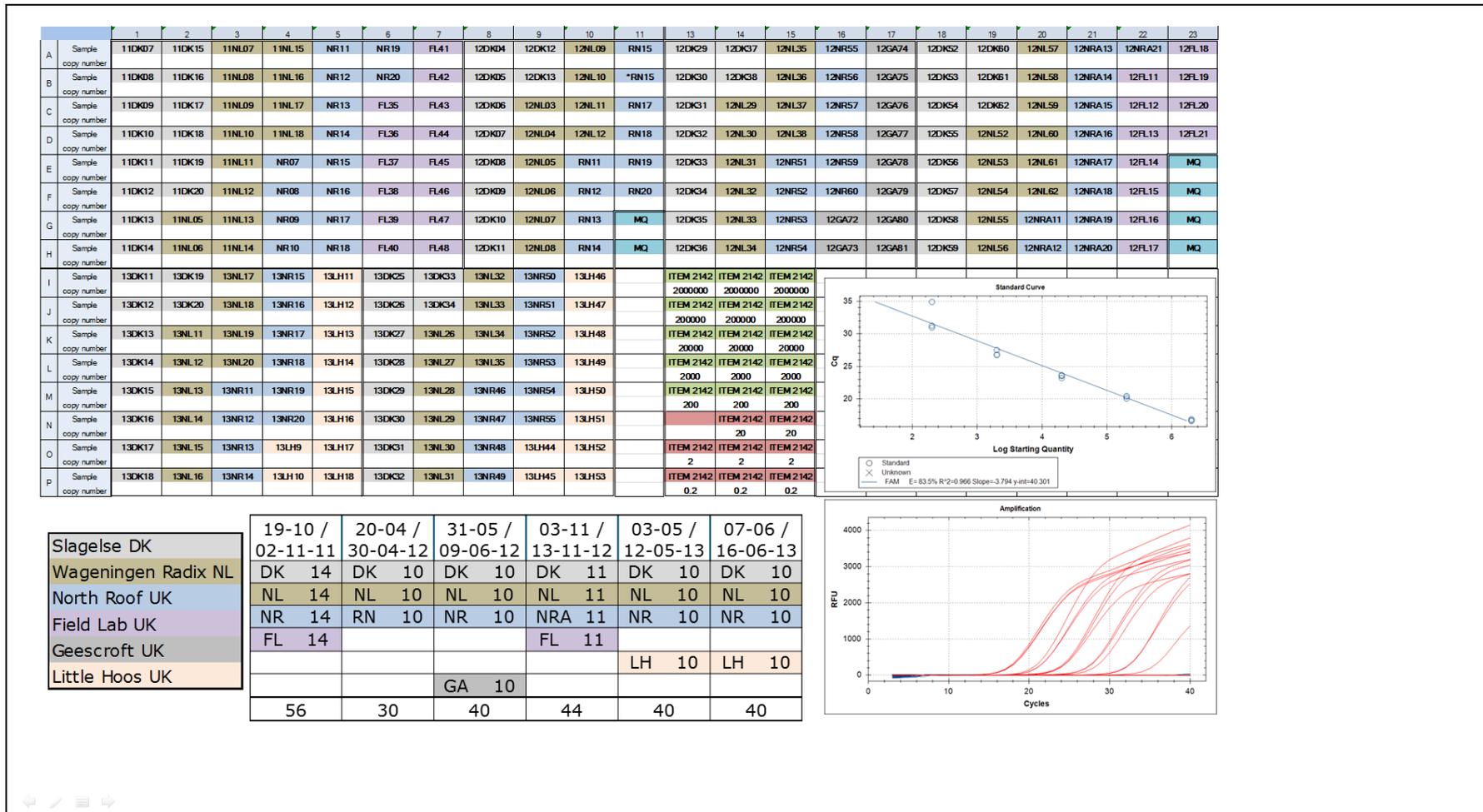


Table 10. Analysis and quantification of the 240 air samples for *Fusarium verticillioides* at different locations. The number of copies is given in the table; empty cells indicate no *Fusarium verticillioides*, numbers indicate the amount of copies of the target DNA in the sample.



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	1	2	3	4	5	6	7	8	9	10	11	13	14	15	16	17	18	19	20	21	22	23	
A	Sample copy number	11DK07	11DK15	11NL07	11NL15	NR11	NR19	FL41	12DK04	12DK12	12NL09	RN15	12DK29	12DK37	12NL35	12NRS5	12GA74	12DK52	12DK60	12NL57	12NRA13	12NRA21	12FL18
B	Sample copy number	11DK08	11DK16	11NL08	11NL16	NR12	NR20	FL42	12DK05	12DK13	12NL10	*RN15	12DK30	12DK38	12NL36	12NRS6	12GA75	12DK53	12DK61	12NL58	12NRA14	12FL11	12FL19
C	Sample copy number	11DK09	11DK17	11NL09	11NL17	NR13	FL35	FL43	12DK06	12NL03	12NL11	RN17	12DK31	12NL29	12NL37	12NRS7	12GA76	12DK54	12DK62	12NL59	12NRA15	12FL12	12FL20
D	Sample copy number	11DK10	11DK18	11NL10	11NL18	NR14	FL36	FL44	12DK07	12NL04	12NL12	RN18	12DK32	12NL30	12NL38	12NRS8	12GA77	12DK55	12NL52	12NL60	12NRA16	12FL13	12FL21
E	Sample copy number	11DK11	11DK19	11NL11	NR07	NR15	FL37	FL45	12DK08	12NL05	RN11	RN19	12DK33	12NL31	12NRS1	12NRS9	12GA78	12DK56	12NL53	12NL61	12NRA17	12FL14	MQ
F	Sample copy number	11DK12	11DK20	11NL12	NR08	NR16	FL38	FL46	12DK09	12NL06	RN12	RN20	12DK34	12NL32	12NRS2	12NR60	12GA79	12DK57	12NL54	12NL62	12NRA18	12FL15	MQ
G	Sample copy number	11DK13	11NL05	11NL13	NR09	NR17	FL39	FL47	12DK10	12NL07	RN13	MQ	12DK35	12NL33	12NRS3	12GA72	12GA80	12DK58	12NL55	12NRA11	12NRA19	12FL16	MQ
H	Sample copy number	11DK14	11NL06	11NL14	NR10	NR18	FL40	FL48	12DK11	12NL08	RN14	MQ	12DK36	12NL34	12NRS4	12GA73	12GA81	12DK59	12NL56	12NRA12	12NRA20	12FL17	MQ
I	Sample copy number	13DK11	13DK19	13NL17	13NR15	13LH11	13DK25	13DK33	13NL32	13NR50	13LH46												
J	Sample copy number	13DK12	13DK20	13NL18	13NR16	13LH12	13DK26	13DK34	13NL33	13NR51	13LH47												
K	Sample copy number	13DK13	13NL11	13NL19	13NR17	13LH13	13DK27	13NL26	13NL34	13NR52	13LH48												
L	Sample copy number	13DK14	13NL12	13NL20	13NR18	13LH14	13DK28	13NL27	13NL35	13NR53	13LH49												
M	Sample copy number	13DK15	13NL13	13NR11	13NR19	13LH15	13DK29	13NL28	13NR46	13NR54	13LH50												
N	Sample copy number	13DK16	13NL14	13NR12	13NR20	13LH16	13DK30	13NL29	13NR47	13NR55	13LH51												
O	Sample copy number	13DK17	13NL15	13NR13	13LH9	13LH17	13DK31	13NL30	13NR48	13LH44	13LH52												
P	Sample copy number	13DK18	13NL16	13NR14	13LH10	13LH18	13DK32	13NL31	13NR49	13LH45	13LH53												

	19-10 / 02-11-11	20-04 / 30-04-12	31-05 / 09-06-12	03-11 / 13-11-12	03-05 / 12-05-13	07-06 / 16-06-13
Slagelse DK	DK 14	DK 10	DK 10	DK 11	DK 10	DK 10
Wageningen Radix NL	NL 14	NL 10	NL 10	NL 11	NL 10	NL 10
North Roof UK	NR 14	RN 10	NR 10	NRA 11	NR 10	NR 10
Field Lab UK	FL 14			FL 11		
Geescroft UK					LH 10	LH 10
Little Hoos UK			GA 10			
	56	30	40	44	40	40

Table 11. Analysis and quantification of the 240 air samples for *Fusarium poae* at different locations. The number of copies is given in the table: empty cells indicate no *Fusarium poae* , numbers indicate the amount of copies of the target DNA in the sample.

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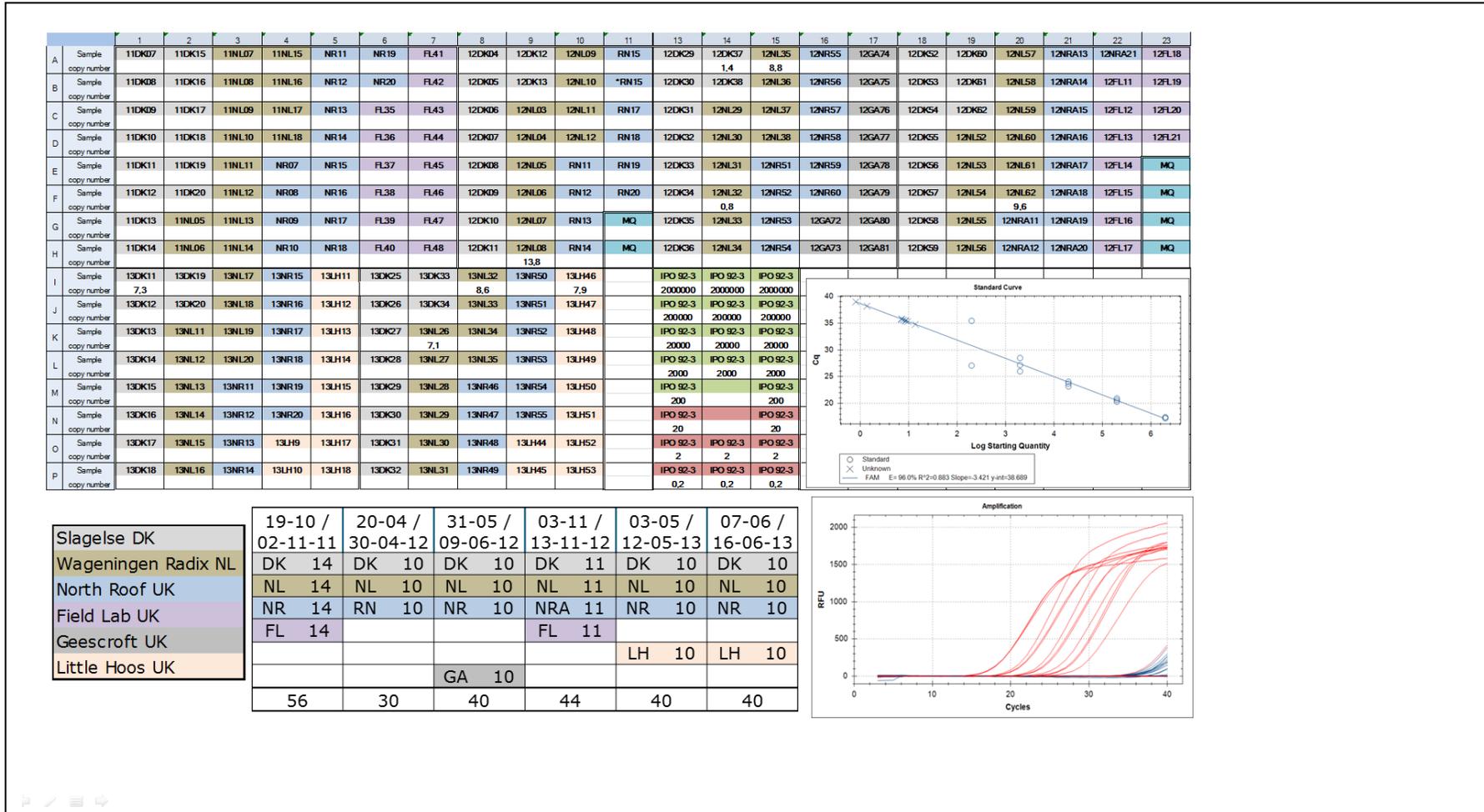


Table 12. Analysis and quantification of the 240 air samples for *Fusarium avenacium* at different locations. The number of copies is given in the table: empty cells indicate no *Fusarium avenacium*, numbers indicate the amount of copies of the target DNA in the sample.

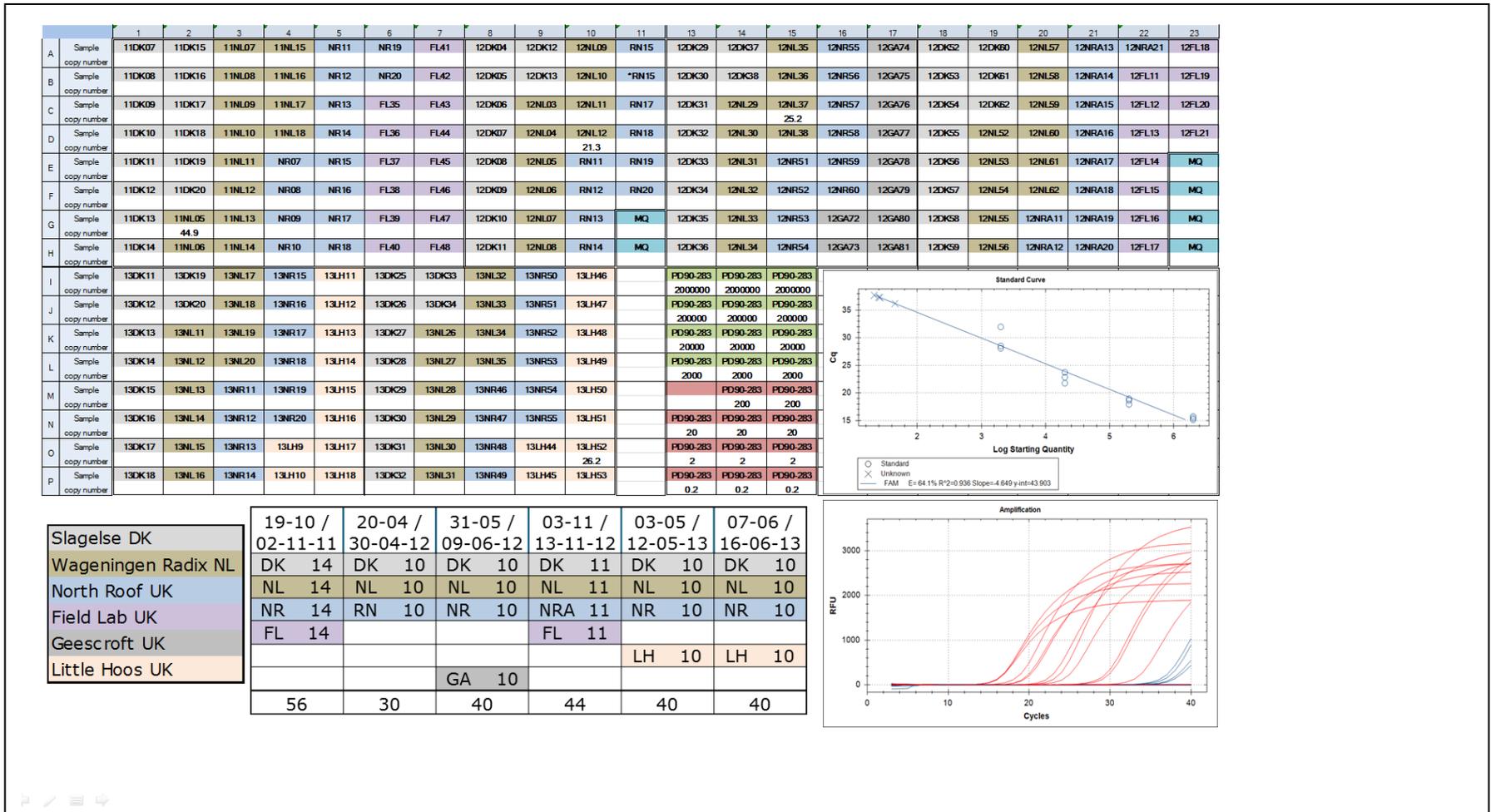


Table 13. Analysis and quantification of the 240 air samples for *Fusarium culmorum* at different locations. The number of copies is given in the table: empty cells indicate no *Fusarium culmorum*, numbers indicate the amount of copies of the target DNA in the sample.

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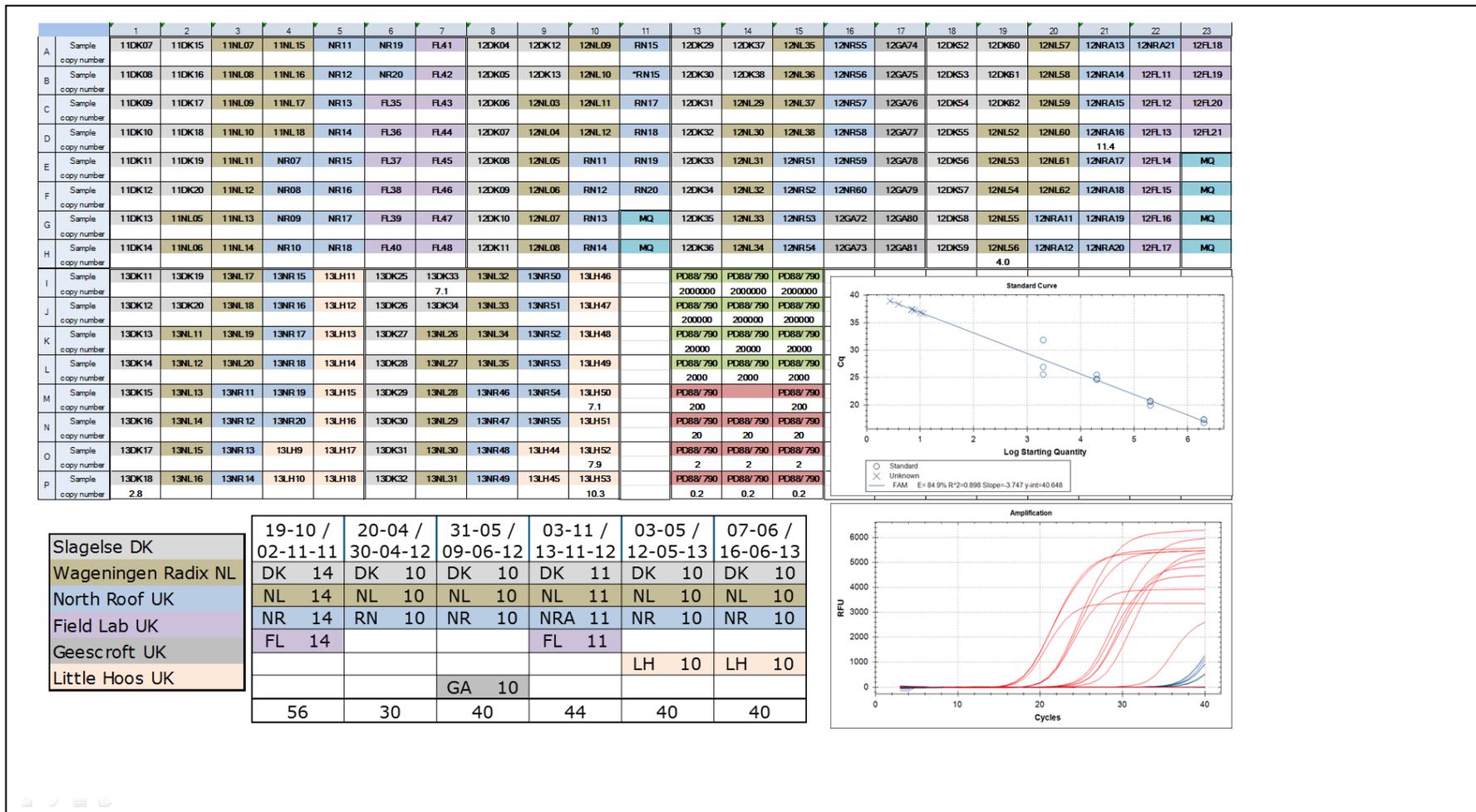


Table 14. Analysis and quantification of the 240 air samples for *Fusarium graminearum* at different locations. The number of copies is given in the table: empty cells indicate no *Fusarium graminearum*, numbers indicate the amount of copies of the target DNA in the sample.

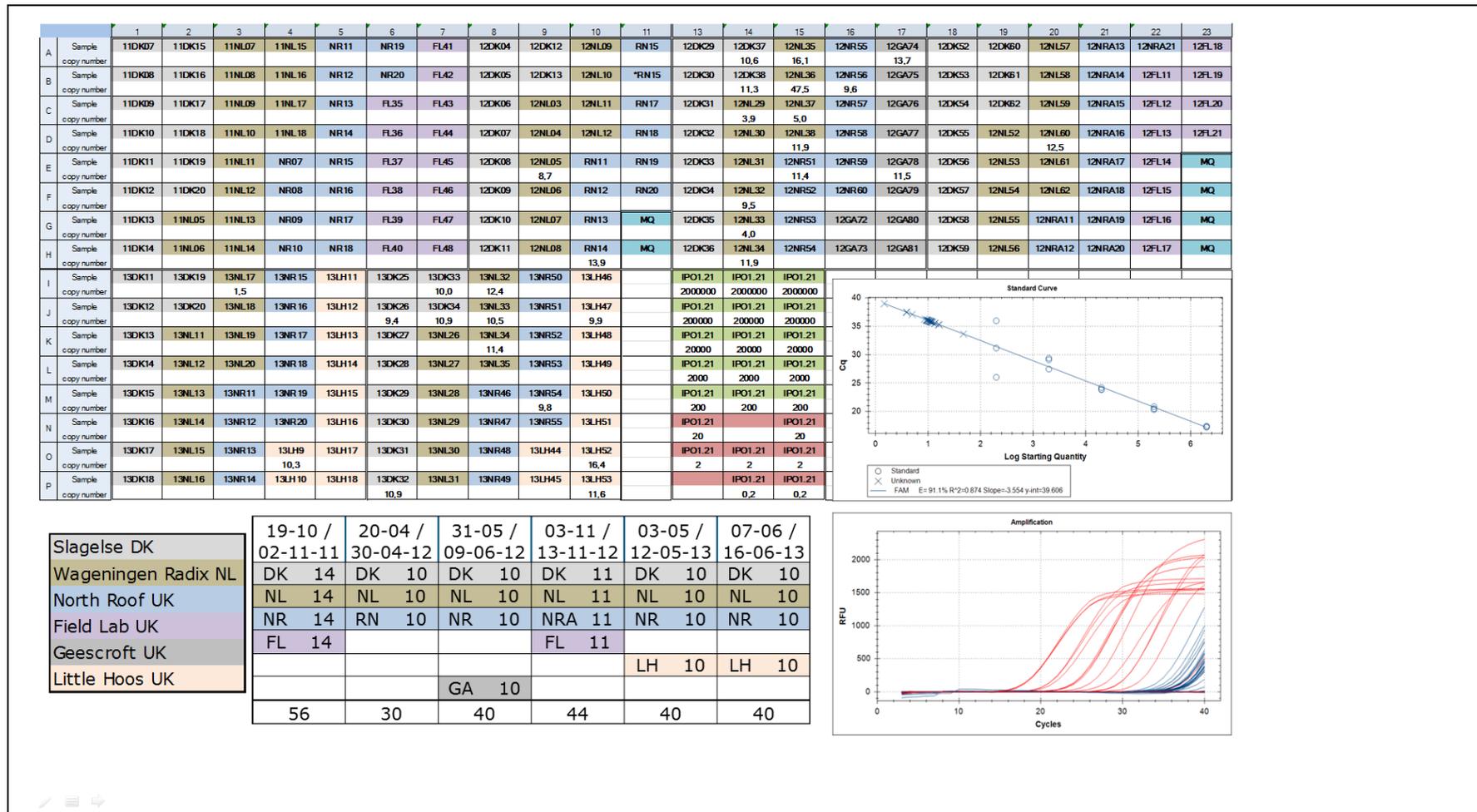


Table 15. Analysis and quantification of the 240 air samples for *Microdochium nivale* at different locations. The number of copies is given in the table: empty cells indicate no *Microdochium nivale*, numbers indicate the amount of copies of the target DNA in the sample

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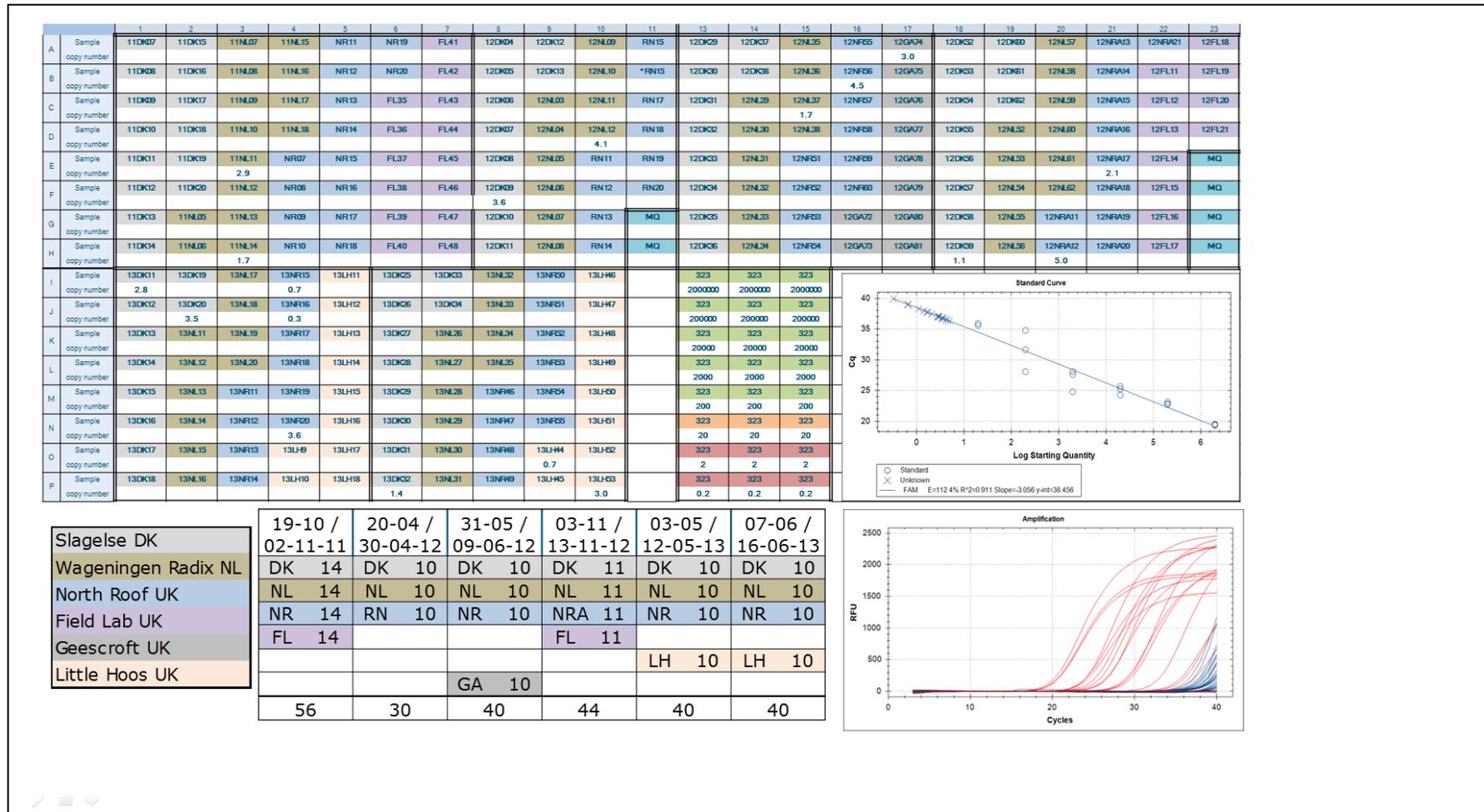


Table 16. Analysis and quantification of the 240 air samples for *Mycosphaerella graminicola* at different locations. The number of copies is given in the table: empty cells indicate no *Mycosphaerella graminicola*, numbers indicate the amount of copies of the target DNA in the sample.

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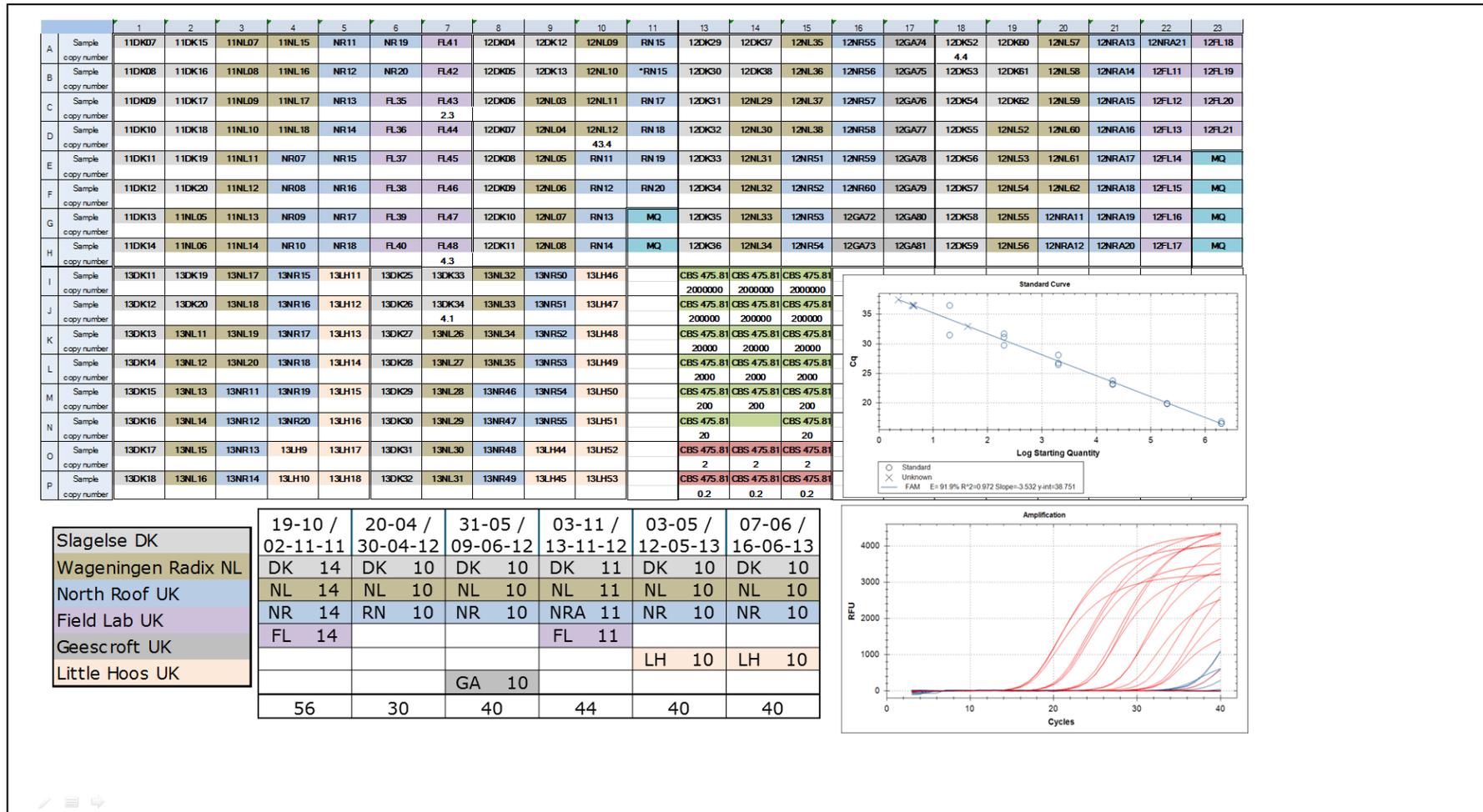


Table 17. Analysis and quantification of the 240 air samples for *Leptosphaeria biglobosa* at different locations. The number of copies is given in the table: empty cells indicate no *Leptosphaeria biglobosa*, numbers indicate the amount of copies of the target DNA in the sample.

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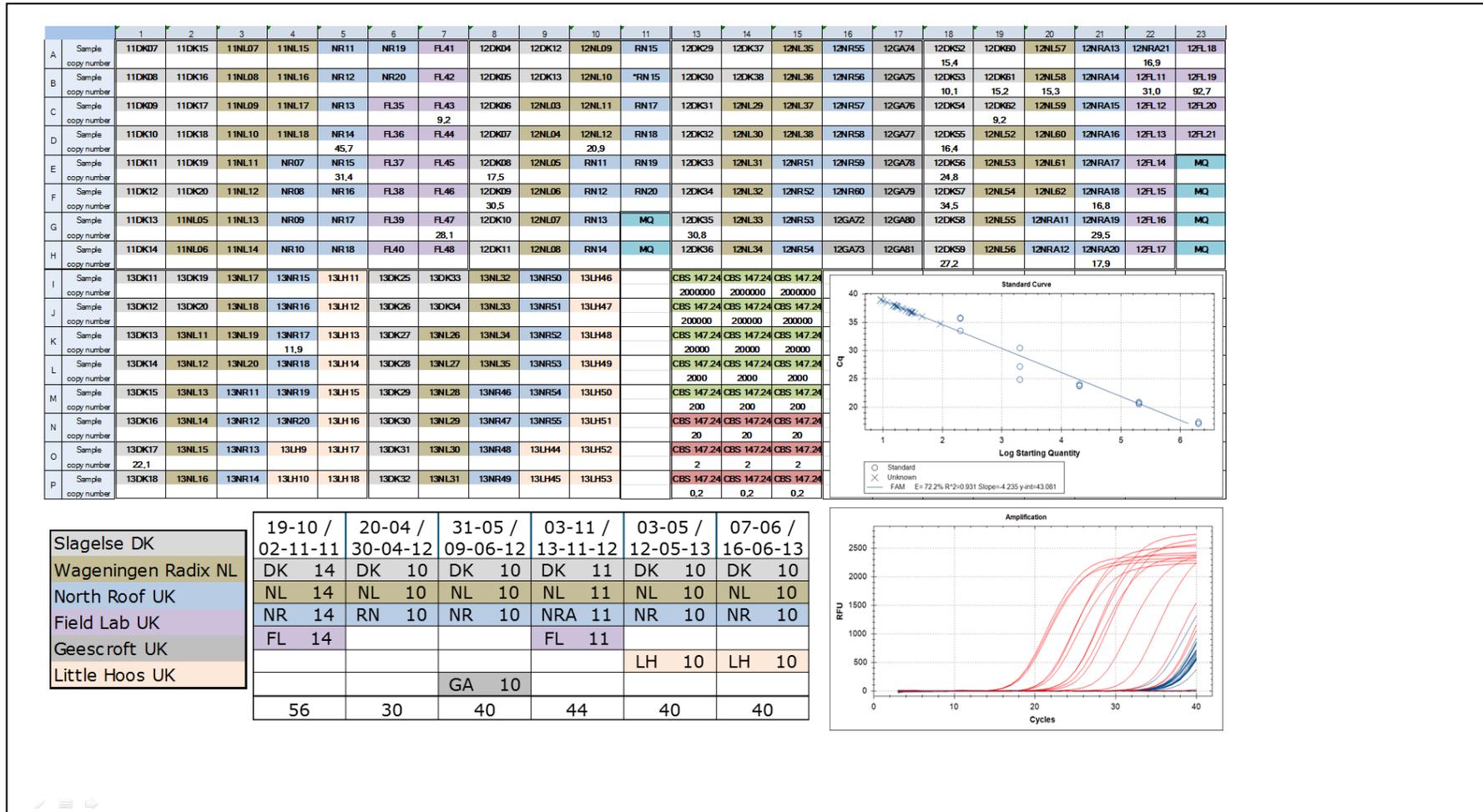


Table 18. Analysis and quantification of the 240 air samples for *Leptosphaeria maculans* at different locations. The number of copies is given in the table: empty cells indicate no *Leptosphaeria maculans*, numbers indicate the amount of copies of the target DNA in the sample.