Golf Course 2030

Sustainable Agronomy

Alternative methods for dollar spot management on sand-based putting greens

Contents.

The Association Francophone Belgian Golf (AFGOLF) is committed to overseeing and promoting golf in Wallonia and Brussels, spanning recreational to high-level play. Its tasks include supervising golf practice, supporting clubs, enhancing the sport's image, fostering inclusivity, improving player skills, implementing sustainable practices, and providing comprehensive training for all stakeholders.

Swiss Golf is the umbrella association for the sport of golf in Switzerland. Its members include 98 golf courses in Switzerland and neighbouring countries, as well as two public golf organisations. It brings together more than 100,000 licensed golfers in Switzerland. In 2018, Swiss Golf initiated a major cultural transformation. Sustainability is now the cornerstone of golf culture in Switzerland. Golf, a sport that fosters social ties, inter-generational exchange and inclusiveness, flourishes in harmony with nature. We take responsibility for the use of water and resources, including plant protection products. Golf can be a sanctuary for people, respecting and preserving the natural environment. Solutions are developed to minimise our footprint, particularly on the greens.

Project supported by:

Introduction.

R&A

1. Introduction

In pursuit of effective solutions for combating cryptogamic diseases, particularly dollar spot, the research featured in this report focuses on evaluating the efficiency of various substances, including fertilisers, bio controls and microorganisms, within the context of pesticide-free management.

The research seeks to evaluate the efficiency of various pesticide-free approaches against dollar spot (*Clarireedia sp.*), testing natural-based substances alongside inorganic and organic amendments to reinforce the plant immunology and fight bio-aggressors on maintained sand-based putting greens.

These substances were tested by means of regular applications on existing greens in Belgium and Switzerland. In parallel, part of the plots was enriched with mineral amendments, such as zeolite and clays, with the objective of assessing their efficacy.

This report outlines the findings of the research, which was conducted on eight golf courses in Belgium and Switzerland. Additionally, the influence of weather conditions, nitrogen inputs and oxygen levels in the soil on disease outbreaks are considered.

2. Research design

This research was designed as a cross-country study, encompassing Belgium and Switzerland, with a total of eight replicates: five in Belgium and three in Switzerland. The research aimed to investigate the efficacy of ten different substances in combating cryptogamic diseases, specifically dollar spot.

Each participating golf course featured putting greens divided into 44 plots of $1m^2$ each, forming a structured experimental setup.

Appendix 1 provides visual documentation of the experimental design, while ***Appendix 2*** offers detailed information about the substances under examination. The detailed table of results of the stastical analysis can be found in ***Appendix 3***.

3. Methodology

3.1 Experimental layout

Each substance was evaluated through a systematic approach, with four $1m^2$ test plots allocated to it. The plots were arranged in 2x2 lines, resulting in two repetitions per putting green. Within each repetition:

- **Both plots received applications of the active substances**
- **One was enriched with mineral soil amendments, including porous ceramics, carbon sources, zeolite and mycorrhizae (referred to as "enriched," Line B).**

Additionally, a 'control' parcel was dedicated on each line, culminating in a total of 44 parcels on each putting green. The randomisation process occurred within each repetition and across different putting greens, ensuring a well-balanced distribution. The greenkeeping team adhered to the specified setup, guided by comprehensive training. The agronomist was responsible for overseeing the implementation and ensuring consistent methodology throughout the entire six-month research period.

3.2 Selection and application of substances

Testing commenced with a series of applications of various substances on both regular and enriched plots. Ten substances were selected for testing, classified into four distinct categories:

- Soil microorganisms
- Natural defence elicitors
- Fertilising elements
- Natural fungal substances.

To maintain impartiality, the greenkeeping team remained unaware of the identity of the products being applied. Products were anonymised and numbered from 1 to 10, with each partnering club receiving precise instructions for product placement on all the parcels. Applications of substances were carried out every two weeks on all plots and the mineral amendments delivered onto the required plots every four weeks.

3.3 Application schedule

The substances were applied at regular intervals:

- Every two weeks, each test plot received a predefined quantity of the assigned substance
- Every six weeks, the 'enriched' parcels were amended with organic soil structure enhancers.

3.4 Duration and maintenance of testing plots

Throughout this period, the test plots underwent regular mechanical maintenance identical to the rest of the course. With regards to all spraying, aside from fertilising products, no pesticides, fungicides, insecticides, biostimulants or other substances were applied to the research plots. All maintenance and spraying activities were diligently documented on a digital platform, with the greenkeeping team providing daily updates, ensuring data accuracy and transparency.

3.5 Data Collection

Oxygen Level Measurement

Regular measurements of oxygen levels available on the greens were conducted on each golf course. A manual oxygen probe was used to assess the percentage of oxygen in the substrate at a depth of around 10-12cm. Measurements were taken monthly.

Weather stations

Weather stations were installed to capture temperature (air and ground), humidity (air and ground), rainfall, dew point, wind speed, solar radiation. From the data, daily ETP (evapotranspiration), DLI (daily light integral) and risk of dollar spot outbreak values were computed each day.

Disease Observation

Disease outbreaks, particularly dollar spot attacks, were closely monitored and documented on each golf course. Throughout the research period, the on-site team took pictures of each 1m² parcel every two weeks. These images were named appropriately, forwarded to AFGOLF, and reviewed, analysed and archived. In addition to visual documentation, the greenkeeping team completed site observation sheets to monitor any abnormalities and document site conditions.

On a monthly basis, the agronomist conducted soil-oxygen readings across each 1m² parcel during their routine visits. The collected data was stored, reviewed and archived. Weather stations and soil sensors, positioned on each participating golf course, facilitated real-time monitoring of local conditions, enriching the dataset.

3.6 Data Analysis

Data collected from weather stations, oxygen measurements and disease observations were analysed using descriptive and quantitative statistics. The influence of weather conditions on disease outbreaks was explored by cross-referencing data. The effectiveness of different substances in preventing disease outbreaks was assessed based on observed outcomes.

3.7 Replication and robustness of results

The strategic replication of the experiment within each putting green, encompassing a variety of climatic conditions and soil profiles, ensured the robustness and relevance of the results at regional, national and international levels. The comprehensive methodology, rigorous execution and comprehensive data collection established a strong foundation for the research findings and its implications.

Figure 1: Application at Wylihof Golf Club – 25-05-2023

Figure 2: Dew removal at Rigenee Golf Club – 18-06-2023

Research set up.

4. Research set up

In March and April of 2023, testing plots were installed across eight golf courses in Belgium and Switzerland:

- **Naxhelet Golf Club, Rigenee Golf Club, Royal Bercuit Golf Club, Royal Hainaut Golf Club and Avernas Golf Club in Belgium**
- **Wylihof Golf Club, Golf Limpachtal and Migros Golfpark Moossee in Switzerland.**

Each golf course taking part in the research received a standardised kit containing substances, dosages and a print-out of the spraying plan, ensuring neutrality in the testing process and limiting risk of errors.

Figure 3: Map of the eight golf courses participating in the research.

Figure 4: Plot set up at Naxhelet Golf Course, Belgium – 01-04-2023

Figure 5: Plot set up at Naxhelet Golf Club, Belgium – 11-04-2023

	1 metre	1 metre	1 metre	1 metre	1 metre	1 metre	1 metre	1 metre	1 metre	1 metre	1 metre
TA	P2A	P1A	P3A	P4A	P9A	P1A+P2A P3A+P4A P5A	P7A	TA	P8A	P1A+P2A P3A+P4A	P10A
TB	P2B	P1B	P3B	P4B	P9B	P1B+P2B P3B+P4B P5B	P7B	TB	P8B	P1B+P2B P3B+P4B	P10B
TC	P3C	P1C	P8C	P4C	P9C	P7C	P10C	P1C+P2C P3C+P4C P5C	P2C	TC	P1C+P2C P3C+P4C
TD	P3D	P1D	P8D	P4D	P9D	P7D	P10D	P1D+P2D P3D+P4D P5D	P2D	TD	P1D+P2D P3D+P4D

TA	randomisation 1
TB	randomisation 1 + amendement
TC	randomisation 2
TD	randomisation 2 + amendement

Figure 6: Randomisation table provided to each golf course

Figure 7 (left): Installation of connected weather station and soil sensor at Wylihof Golf Club , Switzerland – 17-03-2023

Figure 8 (right): Research methodology training at Wylihof Golf Club, Switzerland – 17-03-2023

Figure 9 (below): Second application at Wylihof Golf Club – 22-05-2023

Start of testing.

5. Start of testing

The research aimed to span a complete golfing season, commencing from April to October encompassing six months.

Due to supply issues and unseasonably cold weather at the end of April/early May 2023, the testing commenced with a slight delay compared to the schedule, as native bacteria may only be applied at soil temperature above 10°C. These thresholds were met in May 2023, with testing continuing until mid-November of that year.

6. Weather and disease observations

The weather conditions during the spring and early summer of 2023 were characterised by low humidity and minimal dew, which hindered the establishment of dollar spot outbreaks. Among the eight golf courses, only three reported dollar spot attacks.

By September, all courses had experienced some level of dollar spot, but the damages remained superficial on some sites. In Switzerland, while one green was affected from early May, the others were far less damaged and only experienced some disease pressure from the end of August. In Belgium, a first attack was observed on one site in May, followed by a second attack at a second site later in June. The other three test sites remained disease free for most of the trial or with some light damage observed from the end of August onwards.

Figure 10: Photo taken on the testing green at the start of the trial, Naxhelet Golf Club – 18-05-2023

Figure 11: Photo taken on the testing green during the trial (mid-trial), Naxhelet Golf Club – 10-08-2023

Figure 12: Iron sulphate parcel post dollar spot attack, Naxhelet Golf Club – 10-08-2023

Figure 13: Control (no spray) parcel post dollar spot attack, Naxhelet Golf Club – 10-08-2023

Figure 14: Iron sulphate parcel post dollar spot attack, Rigenee Golf Club – 03-09-2023

Figure 15: Control (no spray) parcel post dollar spot attack, Rigenee Golf Club – 03-09-2023

Results.

7. Results

Over the research period of 27 weeks from 3 May 2023 to 10 November 2023, a total of 4293 observations and 1831 “non-zero disease” observations were collected across a total of 384 m² test plots including 36m2 of control plots.

7.1 Quantitative analysis

7.1.1 Active substances

As illustrated in Figure 16, an initial comparative analysis of the 4293 data points reveals some initial trends, with 9 out of 17 substances demonstrating a reduction in disease severity compared with the control plots, suggesting a certain efficiency of:

- **N fixating bacteria (A)**
- **Iron sulphate (B)**
- **Mix of indigenous bacteria (C)**
- **Mix of indigenous bacteria enriched with Bacillus (E)**
- **Siderophore producing bacteria (F)**
- **P solubilising bacteria (H)**
- **Mixture of lactobacillus / Bacillus natto bacteria (O)**
- **Trichoderma atrobruenneum (P)**
- **Mychrorize (Q)**

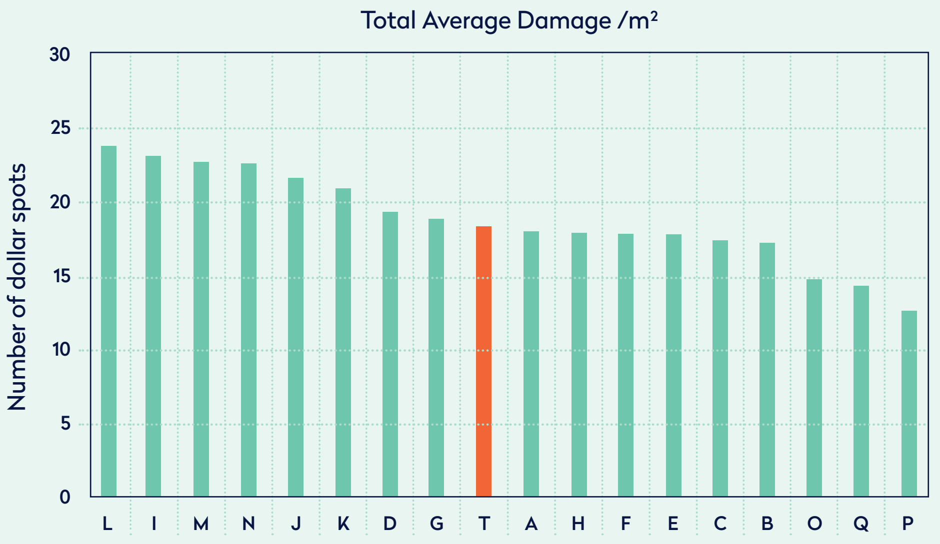

Figure 16: Average disease damage (all blocks)

Statistical analysis.

7.1.2 Amendment analysis

On the other hand, the results for the amendments (organic soil structure enhancers) showed that the disease was more resistant on the amended plots in 40% of cases, suggesting mixed effectiveness, as illustrated in Figure 17.

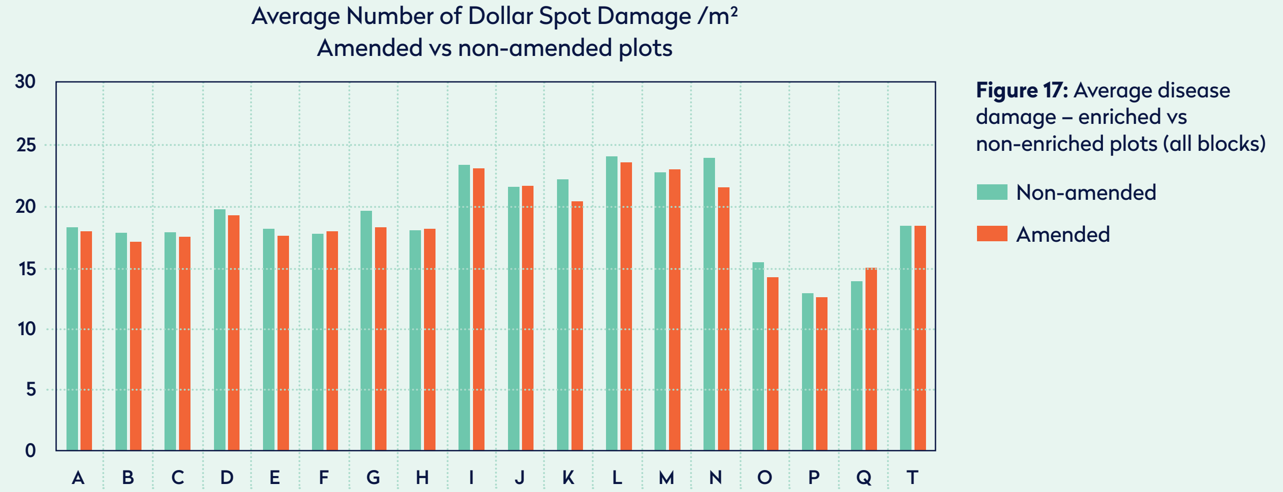

Figure 17: Average disease damage – enriched vs non-enriched plots (all blocks)

Figure 18: Parcel set up at Avernas Golf Club – 26-04-2024

8. Statistical analysis

8.1 Observations and data processing

An initial inspection of the data histogram and its data density curve reveals, as illustrated in Figure 19, the significant bimodal nature of the data, probably due to the long period without pathological events during the trial causing the data to be scattered.

While the Shapiro-Wilk test[1] test suggests a degree of normality, the Levene's test of homogeneity of variances is violated.

Since the variance appears to be proportional to the mean, as shown in Figure 20, a square root transformation was applied to stabilise the variance.

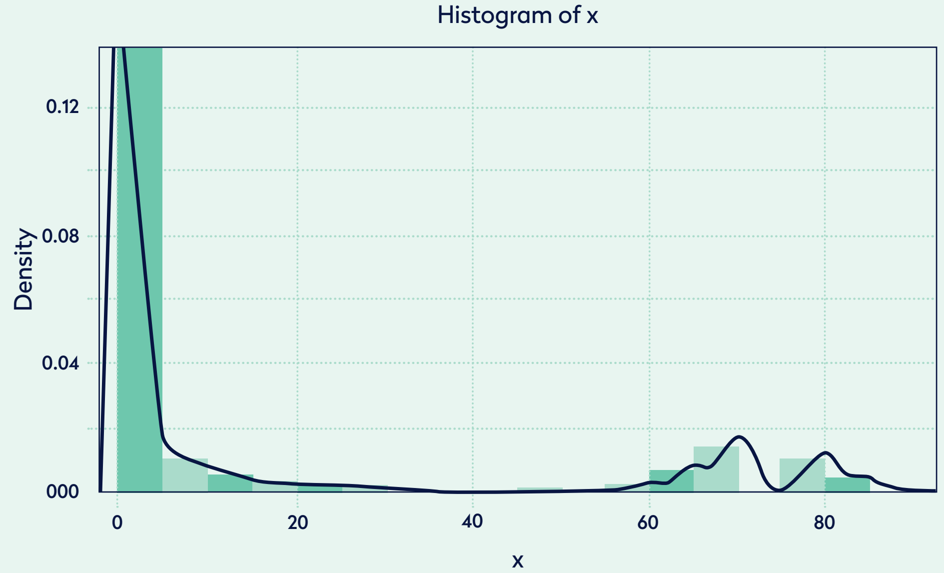

Figure 19 Histogram of raw data density

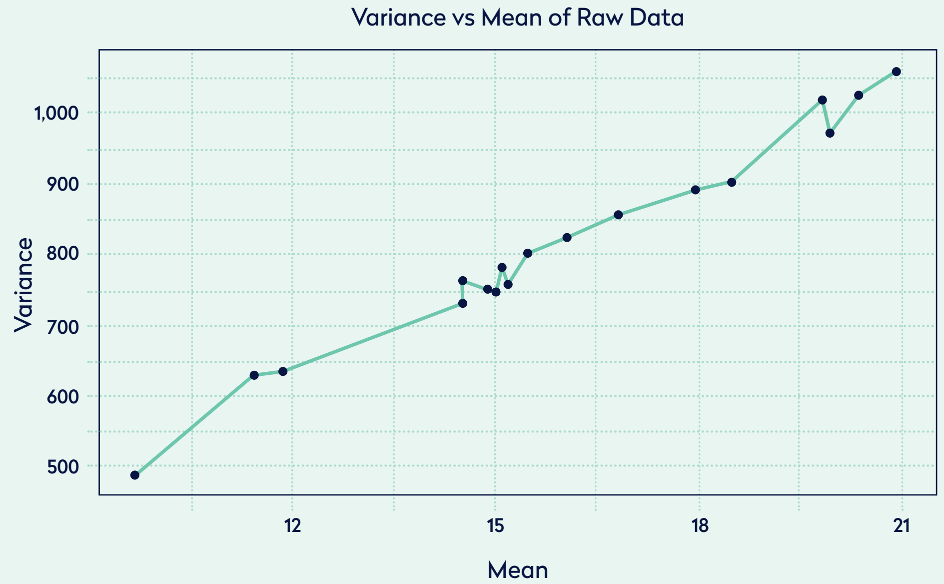

Figure 20: Variance over mean of raw data

Subsequent Shapiro-Wilk test[1] testing on the square root transformed data indicate compliance with normality and enable variance stability with satisfying results from the Levene's test[2].

For comparison, a log transformation was also applied. The square root transformation was deemed more appropriate given the obtained improved fit in comparison to the log transformation, which did not capture as clearly the underlying trends and variability in the data.

With a minimum interval of two weeks between all the data points, the observations were treated as independent events.

[1] **The Shapiro-Wilk test** is a statistical test that assesses the normality of a dataset. It tests the null hypothesis that the data was drawn from a normally distributed population. A significant test result suggests that the data deviates from normality, which can impact the validity of statistical tests that assume normal distribution.

[2] **The Levene's test** examines the homogeneity of variances across groups. It tests whether different samples have equal variances, an assumption required for various statistical tests such as ANOVA. Violation of this assumption can lead to incorrect conclusions, making the Levene's test crucial for validating the conditions for further analysis.

8.2 ANOVA models

An ANOVA (Analysis of Variance) model, applied to the original and transformed data, was used to assess the effects of time (period), management practices (amendment application) and type of substance (product category) on plant resistance to dollar spot.

8.2.1 Effects of the weather

Unsurprisingly, the models highlighted the significant impact of time on all data sets, highlighting the clear seasonal effect of the appearance of dollar spot.

8.2.2 Product category and modification factor

While most product categories did not show any statistically significant effect, product categories A, B, C, E, F and H consistently showed a lower number of illnesses compared to the control. A statistically significant negative effect was found on the pairwise analysis of the square root transformed data for product categories B and C. This points to the potential of these substances to effectively reduce disease incidence and correspond, respectively, to iron sulphate and the mix of indigenous bacteria.

Pair-wise comparison, often performed after an ANOVA test, is a statistical method used to determine whether the differences between the means of two groups are statistically significant. *Appendix 3* includes the detailed table of results.

It is worth noting that the lack of statistical significance in the other categories is most likely caused by the asymmetric nature of the data and predominance of 'non-event' occurrences masking any potential effect(s).

8.2.3 Amendments

According to the ANOVA analysis, the amendments (organic soil structure enhancers) showed no significant impact, suggesting that this factor may not influence the onset of the disease or that the data did not adequately capture their potential effects.

8.2.4 Importance and implications

The significance of the negative effects of products B and C is a crucial finding suggesting that these substances could be beneficial in dollar spot management. However, it is also important to note the limitations imposed by the asymmetry and over-dispersion of the data caused by the prevalence of 'non-event' data points following the relatively dry season, which were not very conducive to dollar spot.

9. Environmental data analysis results

Closer inspection of the data suggests the importance of the environmental context in which the plot exists in determining its fate. Three major areas of influence were identified, as follows.

9.1 Nutritional reserve & nitrogen supply

A closer look at the different nutrition plans for the various golf clubs and courses highlights the relevance of nitrogen input programme in terms of plant disease resistance.

Observing Figure 21 and considering the three sites which were the least and the three sites which were the most impacted by the disease, we can see that the nutrition curve of the most damaged sites shows a systematic drop or misalignment to the growth curve. In comparison, the least attacked sites seemed to have maintained, overall, a larger and more constant nitrogen reserve over the season, thereby avoiding falling into a nitrogen deficit zone.

A more detailed comparison of the nitrogen balance of the different sites can be found in *Appendix 2*.

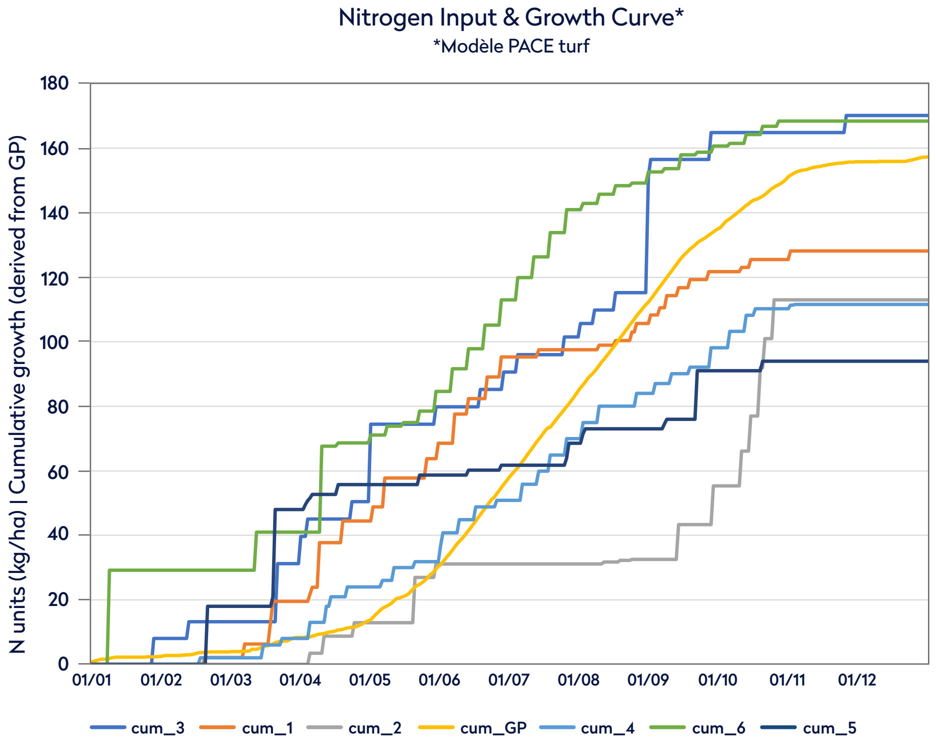

Figure 21: Cumulative nitrogen input and cumulative growth curve based on the PACE turf growth model

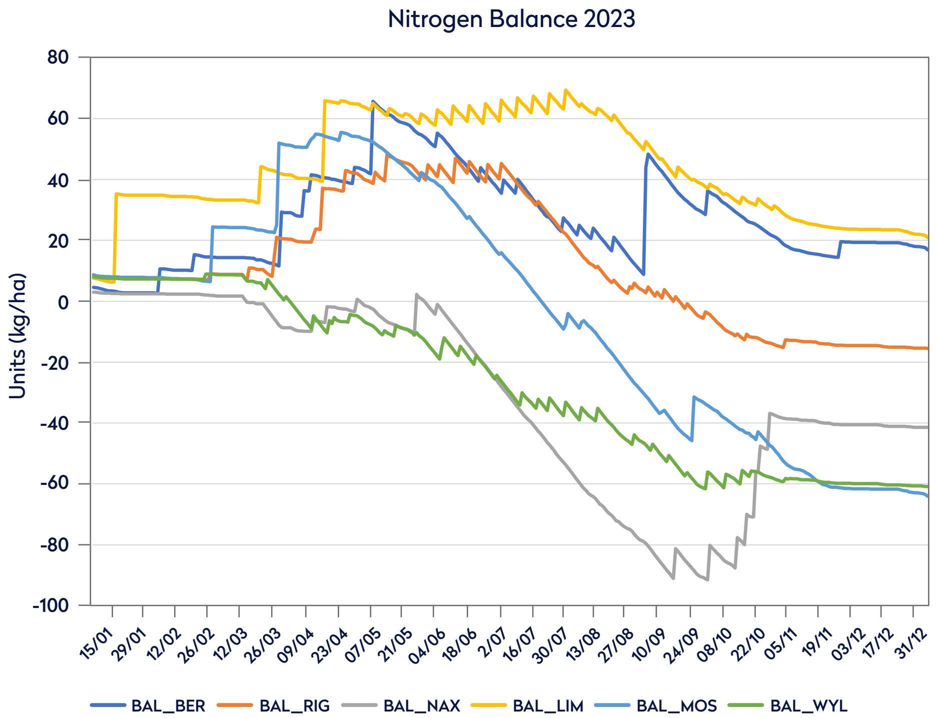

Figure 22: Nitrogen balance across the season based on the PACE turf growth model

A comparison of dollar spot pressure rates using the Smith-Kerns model supports these observations. Whilst disease outbreaks differ greatly between sites, differences in cryptogamic pressure vary little between the different blocks.

In addition to providing the plant with an adequate support and living environment via the soil, two factors seem decisive here. Firstly, ensuring that the plant's reserves are maintained at a sufficient level seems essential. Secondly, it is important to adjust the supply of nutrients to suit the local course condition in terms of grass coverage, sward composition and substrate structure and consider exposure to wind and shade, which will have a major influence on the leaf and its growth activity through the influence of temperature.

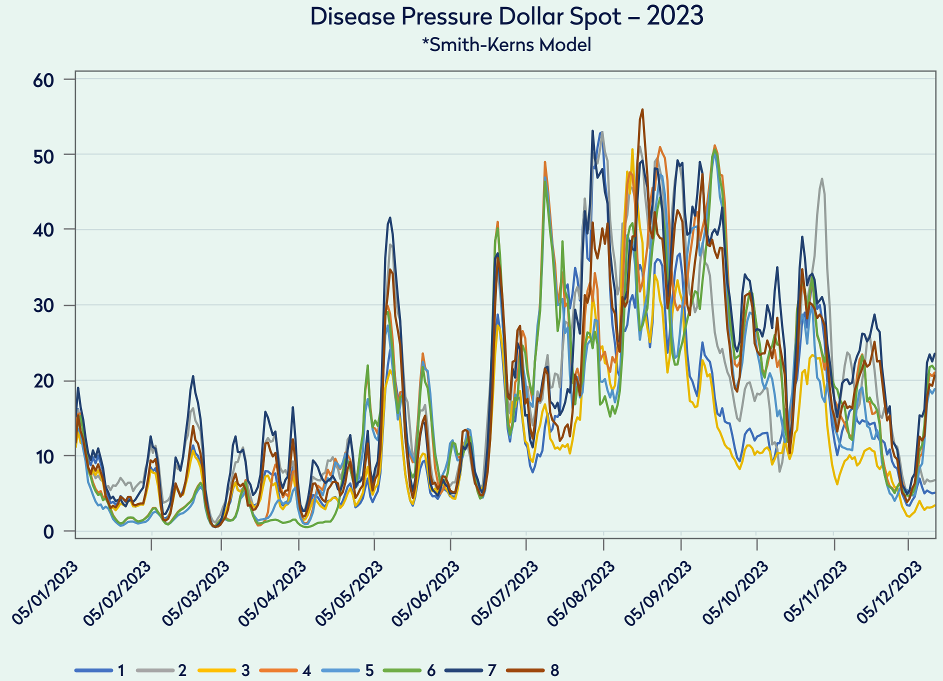

Figure 23: Dollar spot risk according to the Smith-Kerns model for the eight different sites

Whilst sharing extremely similar growth curves, blocks 1, 4 and 5 show distinct nitrogen balance curves and yet demonstrate very good resistance to disease by maintaining, in their own way, a positive nitrogen balance, thereby avoiding falling into a nitrogen-deficient zone and respecting their local growth curve. By comparison, in blocks 2, 3 and 6, a drop in the nutrition curve is systematically observed at some point in the season and disease damages seem to follow systematically shortly after.

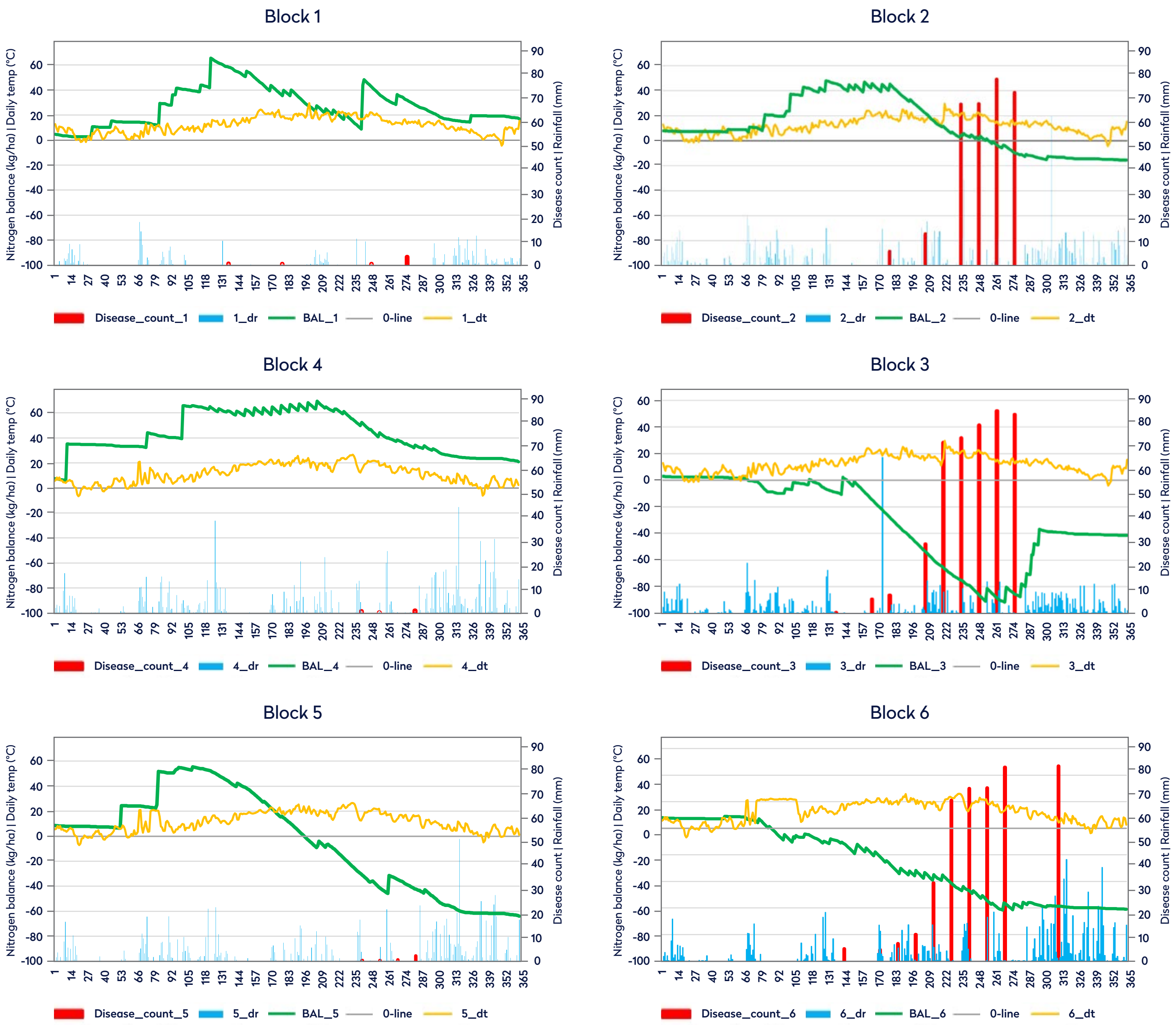

Figure 24: Nitrogen balance curve with disease intensity, air temperature and daily rainfall

9.2 The importance of soil

A closer analysis of the data from the most heavily damaged sites highlights important heterogeneity in product efficacy amongst sites and suggest the significant influence of soil health on the substance's efficacy.

Whilst on some blocks the efficacy of the substances rose to over 60%, in other cases efficacy completely disappeared. As shown in Figure 25, whilst blocks 1, 2 and 6 show good efficacy of the substances, in block 3, no difference seems to be observed between the plots.

The sites express extremely similar sward composition, soil analysis and root profiles. Whilst this trend cannot be explained by any difference in sward composition, which is comparable across the 3 blocks, soil analysis and root profiles reveal a significant difference between the blocks especially in terms of CEC (caution exchange capacity) and OM (outer membrane) level. While block R and W share CEC levels between 25 and 40 and organic matter levels between 1.3 and 2%, block C analysis reports a CEC below 20 and an organic matter level lower than 1%. Exact values can be seen in Table 1.

Block	CEC (meq/kg)	OM (%)
2	38	1.92
3	16	0.76
1	27	1.35
6	29	1.64

Table 1: CEC and OM level for block 1,2,3 and 6

Figure 25: Substances efficacy within different blocks

9.3 Redox, air, water and soil total porosity

Finally, the season highlights the importance of soil balance not only on a chemical level but also on a physical level which is captured by the air/water balance present in the soil.

By collecting over 350 data points on 36 different greens and rating each green's resistance to disease on a scale from 1 to 3, the air/water balance of the substrates was observed. Averaging five measurements on each green across the first 10cm of the substrate, initial observations suggest a strong correlation and indicates a comfort zone within the soil structure in terms of air/water ratio when it comes to good disease resistance, as demonstrated by Figure 26.

The observations shown in the graph may suggest that maintaining a certain volume of air in the soil is more important for disease resistance than the total amount of porosity. In fact, for smaller total porosities, resistance can be just as good if not better, provided the plant receives an adequate supply of oxygen.

A comparison of these results with the blocks of replicates supports this hypothesis, where no direct correlation is observed between total porosity and disease resistance. However, the notion of balance and the ratio between water and oxygen levels seems to play a major role and further investigation should be completed to confirm this first hypothesis.

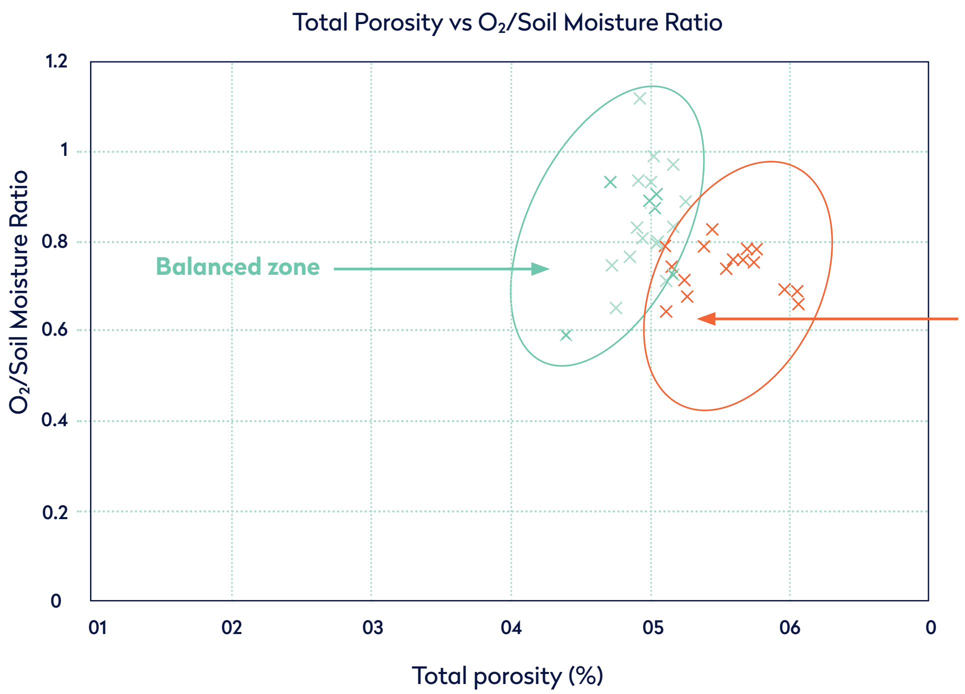

Figure 26: Scatter plot of measured total porosity against the O_2/soil moisture ratio

9.3.1 Effects of the weather

Total porosity

Total porosity refers to the fraction of the soil volume that is not occupied by solid particles, i.e., the pore spaces that can be filled with air or water. In sandy soil, porosity is generally high due to the larger size of the sand particles and the low water retention capacity.

Air/Water balance

The balance between air and water in the soil is essential for root health and plant growth. Roots require oxygen for respiration, and a well-aerated soil provides an adequate supply. Water is needed to transport nutrients, but too much can lead to a lack of oxygen, causing anaerobic conditions that can favour certain root pathogens and diseases.

9.3.2 Understanding the research results and hypothesis

An optimum air/water ratio in the soil ensures adequate oxygen availability for the roots.

If the soil is too saturated with water (due to low air porosity), this can limit the oxygen supply, leading to anaerobic respiration and the production of ROS (reactive oxygen species).

Conversely, an excess of oxygen can also increase the production of ROS at root level, and the over-oxidation of the plant, which would encourage the development of harmful pathogens by pushing the plant into an oxidation zone.

It is also important to note that water stress, either through excess water (waterlogging) or lack of water (drought), can induce oxidative stress. ROS are generated in response to stress, and if the plant cannot eliminate them effectively, this can lead to damage and reduce the plant's ability to defend itself against invaders and diseases. The OM acts as a buffer under oxidative stresses.

Figures 27 & 28: Measurement of soil oxygen level *(Soil oxygen meter, Eijkelkamp)* at Rigenee Golf Club, May 2023

10. Conclusion

The research outlined within this report underscores the efficacy of seven substances, particularly iron sulphate and indigenous bacteria, in supporting plant immunity and naturally lowering dollar spot pressure. It emphasises the critical role of a robust soil microbiome in ensuring the long-term success of Integrated Pest Management (IPM) strategies, asserting that without a healthy microbial environment, such treatments may prove ineffective or only bringing short sighted effects.

In addition, the study delineates three key influences on plant disease resistance to dollar spot. First, it highlights the relevance of precise nutrient management in controlling disease's progression and severity. Second, it emphasises the importance of achieving an optimal air-to-water ratio in the soil, which should be finely tuned according to the specific total porosity characteristics of the location to enhance disease resistance. Additionally, the findings suggest that soils richer in agronomic content may inherently offer stronger disease resistance. Yet, it is crucial to balance this benefit against the need to maintain the satisfying playability of greens, ensuring that improvements in turf health translate effectively to the quality of the golf course and not compromise the golfing experience.

Future strategies should integrate these insights, promoting sustainable management practices that align with ecological and recreational demands.

11. Further research and work

Further research should explore the long-term effects of the identified effective substances and amendments under different environmental conditions to validate and refine the findings.

Investigating alternative substances and integrated management practices could also offer new insights into sustainable disease management strategies.

Future studies might also examine the interactions between soil microbiome diversity and plant health, offering a holistic approach to understanding disease dynamics.

12. Lead research and support team

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Jean-Luc Gavroye, AFGOLF President
All greenkeepers and their greenkeeping team

13. Glossary

ANOVA (Analysis of Variance)

A statistical method used to compare the means of three or more samples to understand if at least one sample mean is different from the others.

Biocontrol agents

Organisms, such as certain bacteria and fungi, used to control pest populations and disease.

Dollar spot

A common turfgrass disease caused by the fungus Sclerotinia homoeocarpa, characterised by small, round, sunken spots on grass leaves.

Elicitors

Substances that stimulate a plant's natural defence mechanisms against pathogens.

Homogeneity of variances

An assumption in statistics that the variances among groups are equal.

Levene's Test

A statistical test used to assess the equality of variances for a variable calculated for two or more groups.

Mycorrhizae

Fungi that have a symbiotic relationship or association with the roots of many plants.

Nitrogen fixation

The process by which the relatively non-reactive nitrogen in the Earth's atmosphere is converted in soil into ammonia or other related nitrogenous compounds available for plants to absorb.

Randomisation

The process of randomly assigning subjects or treatments to groups in an experiment to reduce bias.

Shapiro-Wilk Test

A test that evaluates whether a sample comes from a normally distributed population.

Smith-Kerns Model

A logistic-based dollar spot prediction model that uses a 5-day moving average of daily relative humidity and daily average air temperature to create a probability that dollar spot will occur on a given day.

Statistical significance

The likelihood that a relationship between two or more variables is caused by something other than random chance.

Variance

A measure of the dispersion of a set of data points around their mean value.

Appendices.

14. Appendices

Appendix 1

Overview of the research plots

BELGIUM

HETEROGENEITY PEDOCLIMATIC

BLOC 1 (GOLF 1)	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
	P5	P7	P1	P2	P10	P4	P3	P12	P8	P9	P11	P6
	P5	P7	P1	P2	P10	P4	P3	P12	P8	P9	P11	P6
BLOC 2 (GOLF 2)	P6	P11	P9	P8	P12	P3	P4	P10	P2	P1	P7	P5
	P6	P11	P9	P8	P12	P3	P4	P10	P2	P1	P7	P5
	P2	P1	P3	P8	P10	P9	P2	P11	P12	P5	P6	P7
	P2	P1	P3	P8	P10	P9	P2	P11	P12	P5	P6	P7
BLOC 3 (GOLF 3)	P2	P1	P3	P4	P9	P6	P11	P7	P12	P8	P5	P10
	P2	P1	P3	P4	P9	P6	P11	P7	P12	P8	P5	P10
	P3	P11	P8	P4	P9	P7	P1	P10	P6	P2	P12	P5
	P3	P11	P8	P4	P9	P7	P1	P10	P6	P2	P12	P5
BLOC 3 (GOLF 4)	P7	P6	P5	P12	P11	P2	P9	P10	P8	P3	P1	P2
	P7	P6	P5	P12	P11	P2	P9	P10	P8	P3	P1	P2
	P10	P7	P8	P12	P7	P11	P6	P9	P4	P3	P1	P2
	P10	P7	P8	P12	P7	P11	P6	P9	P4	P3	P1	P2
BLOC 5 (GOLF 5)	P5	P12	P2	P6	P10	P1	P7	P9	P4	P8	P11	P3
	P5	P12	P2	P6	P10	P1	P7	P9	P4	P8	P11	P3
	P1	P9	P8	P6	P11	P7	P8	P10	P8	P12	P2	P5
	P1	P9	P8	P6	P11	P7	P8	P10	P8	P12	P2	P5

SWITZERLAND

HETEROGENEITY PEDOCLIMATIC

BLOC 6 (GOLF 1)	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
	P5	P7	P1	P2	P10	P4	P3	P12	P8	P9	P11	P6
	P5	P7	P1	P2	P10	P4	P3	P12	P8	P9	P11	P6
BLOC 7 (GOLF 2)	P6	P11	P9	P8	P12	P3	P4	P10	P2	P1	P7	P5
	P6	P11	P9	P8	P12	P3	P4	P10	P2	P1	P7	P5
	P2	P1	P3	P8	P10	P9	P2	P11	P12	P5	P6	P7
	P2	P1	P3	P8	P10	P9	P2	P11	P12	P5	P6	P7
BLOC 8 (GOLF 3)	P2	P1	P3	P4	P9	P6	P11	P7	P12	P8	P5	P10
	P2	P1	P3	P4	P9	P6	P11	P7	P12	P8	P5	P10
	P3	P11	P8	P4	P9	P7	P1	P10	P6	P2	P12	P5
	P3	P11	P8	P4	P9	P7	P1	P10	P6	P2	P12	P5

Detailed view of one plot

	1m	1m	1m	1m	1m	1m	1m	1m	1m	1m	1m	1m	1m	1m	1m
A	P1A	P6A	TA	P1+P2 P3+P4 A	P7A	P2A	P10A	P12A	P1+P2 P3+P4 P5A	P11A	P3A	P13A	P9A	P8A	P4A
B	P1B	P6B	TB	P1+P2 P3+P4 B	P7B	P2B	P10B	P12B	P1+P2 P3+P4 P5B	P11B	P3B	P13B	P9B	P8B	P4B
C	P9C	P4C	P11C	P1C	P6C	P3C	P8C	P1+P2 P3+P4 C	P10C	P2C	TC	P12C	P13C	P7C	P1+P2 P3+P4 P5C
D	P9D	P4D	P11D	P1D	P6D	P3D	P8D	P1+P2 P3+P4 D	P10D	P2D	TD	P12D	P13D	P7D	P1+P2 P3+P4 P5D

	Enriched line, mineral amendments applied (mix of zeolite, porous ceramic, diatomaceous earth, plant organic matter and mycorrhizae)
	No mineral amendments applied

Appendix 2

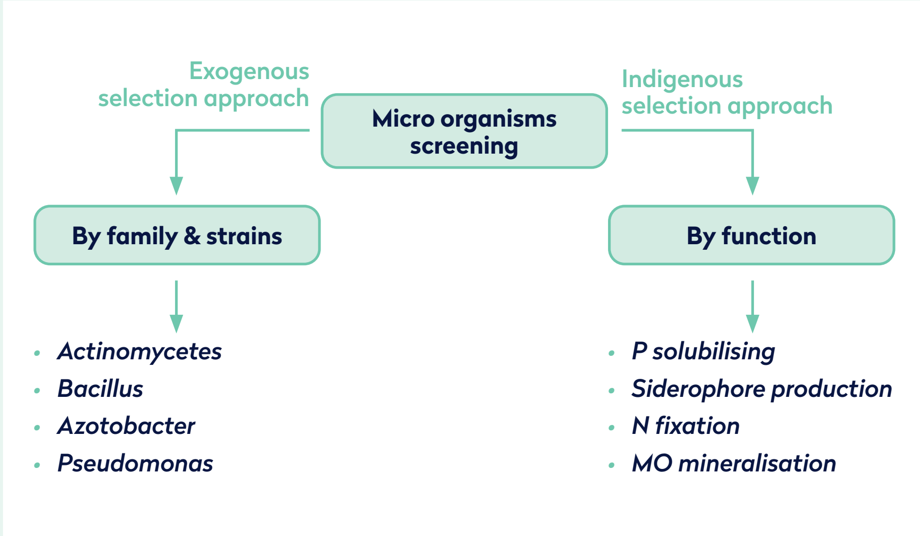
Details of the substances researched

Switzerland variants		Belgium variants	
G	Sulphate ammonium	G	Sulphate ammonium
B	Iron sulphate	B	Iron sulphate
H	P solubilising bacteria (B1)	H	P solubilising bacteria (B1)
D	MO mineralising bacteria (B2)	D	MO mineralising bacteria (B2)
F	Siderophore producing bacteria (B3)	F	Siderophore producing bacteria (B3)
A	Nitrogen fixating bacteria (B4)	A	Nitrogen fixating bacteria (B4)
C	Mix bacteria (B1+B2+B3+B4)	C	Mix bacteria (B1+B2+B3+B4)
E	Mix bacteria with Bacillus spp (B1+B2+B3+B4 + Bac)	E	Mix bacteria with Bacillus spp (B1+B2+B3+B4 + Bac)
O	Lactobacillus – Bacillus natto – yeast fungus	I	Chitosan
P	Trichoderma spp. (atrobrunneum)	K	Coniothyrium Minitans
Q	Mychoryzea	N	Sulphur and manganese
		J	Amino acids (BE only variant)
		M	Willow bark infusion (BE only variant)
		L	Iron EDDHA (BE only variant)

Testing for microorganisms

In terms of testing for microorganisms, the research approach followed the principle of soil bioaugmentation most used in organic agriculture (Kumar et al., 2015) and soil regenerative agriculture.

Rather than choosing a specific exogenous strain of microorganisms to introduce into the soil, this method involves the isolation of naturally occurring bacteria already present in the soil and can perform determined desired functions. This is achieved through a meticulous process of identification, selection and cultivation within a controlled laboratory environment. By recognising the distinctiveness of each soil ecosystem, this approach enables each soil ecosystem the precise isolation of the strains that exhibit the desired functions within the specific soil environment under observation. Ultimately, the method involves reintroducing these isolated native bacteria back into the original site, encouraging the promotion and restoration of a healthy soil ecosystems.



Four functions were focused on: phosphorous solubilisation, siderophore production, fixation of nitrogen and organic matter mineralisation. For each soil sample collected on each site, the bacteria carrying out the function locally were isolated, multiplied and sprayed on to the soil at two-week intervals throughout the research.

In addition to each being pulverised individually, these same strains of bacteria were sprayed together and together with the addition of Bacillus.

An additional parcel was also sprayed with a mix of Trichoderma spp. (atrobrunneum) and another with lactobacillus (these later two were only tested in Switzerland).

A total of eight microorganisms tests were carried out: six with native soil bacteria and two with pre-selected strains.

Natural Plant Defence Elicitors

For the elicitors part, three types of substance were tested: chitosan (for its chitin content), willow bark infusion (for its salicylic acid content) and Coniothyrium Minitans (Didymellaceae family) within the Ascomycota phylum of fungi. These variants were only tested on the Belgian sites.

Nutrients

Considering the effect of fertilising agents, ammonium sulphate and iron sulphate were tested. In addition, in Switzerland, the effect of mychoryzea was also tested and in Belgium, the use of sulphur and manganese as fertiliser agents was also evaluated as local variants.

Reference

Kumar BL, Gopal DV. *Effective role of indigenous microorganisms for sustainable environment. 3 Biotech. 2015 Dec;5(6):867-876. doi: 10.1007/s13205-015-0293-6. Epub 2015 Apr 4. PMID: 28324402; PMCID: PMC4624139.*

Appendix 3

Statistical Analysis Results

Product	Cat	rep	Original Data	p-value	Log transform	p-value	Sqrt transform	p-value	Data inspection (non statistical) Observed effects?
			Analyse ANOVA						
N solubilising bacteria	A	36	-0.37	0.94	0.00	1.00	-0.07	0.79	1
Iron sulphate	B	36	-1.02	0.09	0.00	1.00	-0.15	0.0581*	1
Mix of indigeneous bacteria	C	36	-1.02	0.09	-0.01	0.99	-0.16	0.0252*	1
MO mineralising bacteria	D	36	1.30	0.01	0.01	0.99	0.12	0.20	0
Mix of indigenous bacteria + Bacillus spp	E	36	-0.53	0.77	0.00	1.00	-0.06	0.89	1
Siderophore producing bacteria	F	36	-0.74	0.42	0.00	1.00	-0.10	0.38	1
Ammonium sulphate	G	36	0.73	0.43	0.01	1.00	0.06	0.85	0
P solubilising bacteria	H	36	-0.47	0.86	-0.01	1.00	-0.09	0.59	1
Chitosan	I	24	2.19	<.0001	0.04	0.79	0.20	0.01	0
Amino acids	J	24	0.20	1.00	0.04	0.81	0.03	1.00	0
Coniothyrium Minitans	K	24	-0.28	0.99	0.03	0.94	-0.04	0.98	0
Iron EDDHA	L	24	2.62	<.0001	0.05	0.58	0.29	<.0001	0
WIllow bark infusion	M	24	1.64	0.00	0.05	0.49	0.21	0.01	0
Sulphur and manganese	N	24	1.59	0.01	0.03	0.88	0.13	0.24	0
Lactobacillus – Bacillus natto – Yeast fungus	O	12	0.65	0.86	-0.04	0.78	0.01	1.00	1
Trichoderma spp. atrobrunneum	P	12	-1.35	0.12	-0.05	0.61	-0.18	0.14	1
Mychoryzea	Q	12	0.29	1.00	-0.05	0.64	-0.06	0.98	1

Bioaugmentation (indigeneous bacteria)

Statistically significant

"Negative" effect in comparisons of control

R&A

The R&A group of companies was formed in 2004 to take on The Royal and Ancient Golf Club of St Andrews' responsibilities for governing the Rules of Golf, staging The Open, golf's original championship, and developing the sport. The World Golf Museum in St Andrews is part of The R&A group.

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The R&A has responsibility for running a series of world class amateur events and international matches in women's and girls' as well as men's and boys' golf. The R&A stages the AIG Women's Open and works with the DP World Tour to stage the Senior Open presented by Rolex.

The R&A is committed to investing £200 million over ten years in developing golf and supports the growth of the sport internationally, including the development and management of sustainable golf facilities. For more information, visit www.randa.org.

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