NEM EMERGE

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D10 - PCN Proliferation model

NEM EMERGE

AN INTEGRATED SET OF NOVEL APPROACHES TO COUNTER THE EMERGENCE AND PROLIFERATION OF INVASIVE AND VIRULENT SOIL-BORNE NEMATODES

Proliferation models for emergence of virulence in PCN populations

WP4

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Authors	Misghina Teklu, Leendert Molendijk, Thomas Been (P9 - WR)
Revised and approved by	INRAE, JKI,JHI, WU

Contents

Executive summary

Potato cyst nematodes (PCN) are pathogens of potatoes across Europe. Conventional nematicides (fumigants and non-fumigants) including broad range nematistats provided the main source of management before off-target environmental consequences restricted their use. Breeding programs releasing PCN resistant cultivars have been a major success story, currently providing the best method for PCN control within the growing season. Resistant cultivars, complemented with other management strategies (e.g. sampling methods for early detection and use of decision support systems) allow custom management programmes to be implemented.

Resistant cultivars can reduce population densities based on their Relative Susceptibility (RS). If the RS is 1%, the maximum population density in the field is reduced to 2 eggs (g dry soil)$^{-1}$, the damage threshold limit. In Europe resistant cultivars are categorised into a score 1- 9 based on their RS. Resistant varieties with score 9 are the most resistant, having a relative susceptibility of < 1%.

Surveys from the 'Teelbeschermende Maatregelen' (TBM) foundation demonstrate that after 2012 the number of fields infested with PCN started to increase in starch potato growing areas in The Netherlands after years of decline. Similarly seen in Germany, starch potato fields growing the most resistant starch cultivars (Arvana, Festien, Seresta), displayed locally delayed flowering and tuber setting when grown in a 'normal' low full field infestation. These typical infestation foci indicate the introduction and local build-up of a virulent population. In pot experiments the RS of prominent starch potato cultivars, including Aveka, innovator and Seresta was increased from 2% for the reference *G. pallida* test population (E400) to 30-40% when tested with the virulent field population of *G. pallida* (Oberlangen and Ampop) from Germany and The Netherlands, respectively. Both the Oberlangen and Ampop populations displayed similar levels of virulence. These virulent populations of PCN are widespread and can now be detected through routine observations. However, the oval shape of an infestation focus can be caused by several other abiotic stresses (water logging, localized nutrient deficiency etc.) and must be checked to confirm PCN presence. For this a reliable method based on soil sampling and if possible, by molecular techniques is required to rapidly detect virulent populations.

Management of PCN relies on early detection of their presence, preventing both populations increase and spread. The importance of early detection is vital as once introduced and established, eradication of PCN is not possible (Efsa, 2012). Population dynamics for new virulent populations should be better understood as these can differ from known reference population. Identification of virulent PCN populations will require:

1. A reliable sampling methodology, defined by sample core size, sampling grid, sampling area and known detection probability which provides information on the 'expected population density' of a cultivar on a standard population.
2. The partial resistance of the potato cultivars grown by the farmer, expressed as RS. The EU score cannot be used. However, both scores are produced by the testing agencies.
3. The population dynamic parameters of the reference test population on a susceptible/resistant reference cultivar e.g. Desiree/Seresta.
4. The population dynamic parameters of the virulent population on a susceptible /resistant reference cultivar e.g. Desiree/Seresta.
5. A platform for data storage, making simulations, processing, visualization and decision making.

In view of the above requirements the web-based decision support system in development, NemaDecide Geo+, on the Farmmaps platform is a suitable tool as points 2, 3 and 5 are already covered. Selecting the sampling methods (existing or new ones) and how they perform in detecting significantly higher densities as expected is one part of D10. Extending NemaDecide with the required parameters to detect the emergence of virulent populations of PCN in the field and adding this functionality to the Graphical User Interface (GUI) is a second part of D10.

The details of the input parameters needed and the output in relation to the detection and progression of virulence in PCN will be described and practically demonstrated with examples. The program is expected to help farmers and growers in early detection and containment of virulent PCN population by suggesting appropriate management strategies.

Introduction to the deliverable

NemaDecide is a web-based decision support system (DSS) for the management of plant-parasitic nematodes using applied quantitative nematology data and models generated over the last 60 years. Originally, NemaDecide was developed for the management of PCN. As complete eradication of PCN is not a practical aim, the main objective of NemaDecide as a DSS is to keep population densities below the damage threshold level where no yield losses will be suffered, and redistribution is minimal. The original NemaDecide program ran as executable software on a PC, as a system for advisory services and breeders' organizations. GEO+ is a prototype web-based version developed on the Akkerweb platform to be used by farmers. GEO+ combines sampling data with geographic information allowing the geographical presentation of infested fields and the reconstruction of infestations, providing improved management options and functions to calculate several scenarios simultaneously finding the best management solutions. The program has been ported to the new Farmmaps platform.

The Graphics User Interface (GUI) was closely developed in consultation with advisors and farmers to provide a tool that is easily interpretable by plant protection service experts. To enhance the acceptation of the web-version by farmers, NemaDecide provides an application to request a soil sampling by a sampling agency. The sampling results are returned to the system and are available to the DSS in such a way that no data entry is needed by the farmer. A special application, called Stripbuilder, allows the soil sampling agencies to split fields in any desired number and size of sections required by the sampling method. Maps for the soil sampler and sampling results can now be generated automatically. Based on the population densities farmers can now choose potatoes with different RS and explore different management options.

Apart from sampling data, other input data such as values estimating the effect of host resistance on population development and damage threshold values to predict yield losses are available and stored within the database. With the challenges of emerging virulent PCN populations in Northwest Europe, expansion of NemaDecide to include a function for early detection of virulent populations and their suggested management is required. This is a key deliverable for the NemEmerge project.

In this deliverable NemaDecide will include a proliferation model assisting detecting of virulent populations. Population dynamics and damage threshold parameters should be established based on data available from Dutch and German virulent populations of PCN and can be added later to the data component of NemaDecide. The following data are crucial to be generated for proper simulations and decision making.

1. Selection of an appropriate sampling method for detecting of virulent populations for both full field infestations (starch potatoes) and infestation foci (ware and seed potatoes).
2. Incorporating the required functionality into the NemaDecide code and GUI.
3. Establishing population dynamics for Oberlangen and Ampop populations.
4. Establishing Relative susceptibilities (RS) and Relative Virulence (Rvir) on several selected resistant varieties.

Deliverable description

NemaDecide is a stochastic quantitative DSS. Its infrastructure and input data required are outlined in **Fig.1**. A detailed description of each component of the model will be described in the following sections. Selected examples and illustrations of what the software can do with different management scenarios including the possibility of detecting virulence, will be described.

Major components of NemaDecide on Farmmaps

- The original activeX-component NemaMod®, the engine of the DSS that provides its functionality by incorporating all nematological knowledge – was reconstructed into web-based code that also can be used by third parties using an Application Program Interface (API). It contains all the models to execute the required calculations.
- A set of Dynamic Link Libraries (DLLs) was developed as the core of the Stripbuilder application, which is utilized to incorporate sampling logic and algorithms to divide fields into agronomic areas (sampling units, cropping areas, etc.).
- SQL databases store data related to parameters of the different models for each nematode/host combination, cultivar properties, chemical controls etc. These can be updated or extended with new nematode/host combinations without the need to change the NemaMod® component or NemaDecide itself. This feature makes the engine independent of the GUI and can easily updated to the current state of knowledge concerning parameter values and their stochasticity. This not only extends the use of the component in actual applications (such as new virulence), but also provides the possibility to adapt parameter sets for different countries if required.
- A user interface that gives access to the engine and visualisation components to display model outcomes including calculations, scenario simulations and providing a task map for carrying out management scenarios.

Models used

Models used in NemaDecide are mathematical analogues of nematological theories developed over time span of more than 60 years. The following subjects are covered when using the models:

1. Spatial distribution patterns and sampling methods.
2. Plant growth: yield loss models based on pre-plant nematode densities.
3. Population dynamics of PCN on available hosts.
4. Population declines in the absence of hosts.
5. Relative susceptibility values.
6. The effect of control measures on the parameters of these models.

These parameters have a clear nematological meaning and can be estimated directly from data sets. The models have reliable predictive value and contribute to theory building.

Definition of sampling methods :

TBM sampling methodology: A soil sample of 2400 cc/ha from a grid of 11 x 8 m. In total 120 cores of each 20 cc are collected. A sub-sample of 200 cc is processed from the bulk sample.

EPPO sampling methodology 1: A soil sample of 1200 g /ha from a grid of 10 x 10 m. In total 100 cores of each 15 g are collected. The whole bulk sample is processed for further research.

EPPO sampling methodology 2: A soil sample of 600 g /ha from a grid of 7.5 x 7.5 m. In total 182 cores of each 3.3 g are collected. The whole bulk sample is processed for further research.

AMI100 sampling methodology: A soil sample of 13.9 kg/ha from a grid of 6 x 5 m. In total 331 cores of each 42 g are collected. The whole bulk sample is processed.

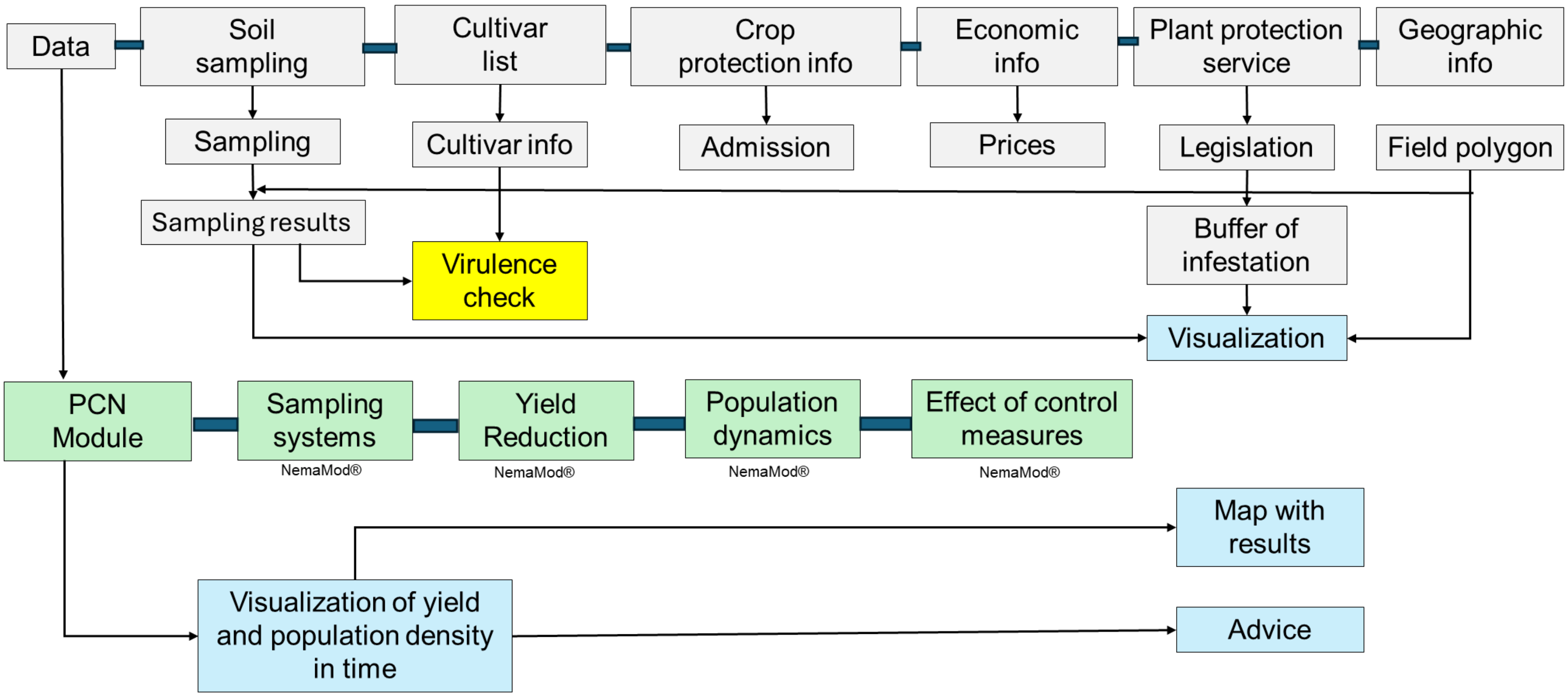

Fig. 1: The structural components of NemaDecide

Spatial distribution patterns

Crop losses and financial returns from nematode control can only be predicted within the limits of nematode field density estimates. The distribution of nematodes in fields is not random, even in small areas. Therefore, sampling practices and sample size must be adapted to the expected small- and medium-scale distribution of nematodes (Been *et al*., 2024).

Small-scale distribution results from aggregation of nematodes around the roots of their hosts when multiplying and the regular pattern in which these host are grown in the field. Medium-scale distribution refers to the size and the shape of an infestation focus which is the result of redistribution of nematodes by farming practices and population dynamics over time. This information can be used to design sampling methods with predictable sampling error and to evaluate current sampling methods. Both the small-scale (1 m^2) and medium-scale (30-500 m^2) distribution patterns of PCN have been extensively studied. In the case of full field infestations there is no area left inside the field without PCN. Infestation foci have grown unchecked, merged into one field-wide infestation with densities dependent on the resistant cultivars used. This happend in starch potato areas, where potatoes can be grown on PCN infested land in 1:2 crop rotations.

Forty fields with infestation foci in five different cropping areas and 10 fields with full-field infestations in the northeastern part of The Netherlands were mapped to model and parameterize the spatial distribution of PCN (Schomaker & Been, 1999; Been & Schomaker, 2000). These models are used in NemaDecide to estimate the variation of initial population (*P*i)-values and enable cost/benefit calculations of various sampling methods.

Plant growth

The relation between (*P*i) and plant weight (*y*) is derived from a growth model for the first mechanism of growth reduction (Seinhorst, 1986b).

$$\frac{r_{Pi}}{r_0} = k + (1-k)\, z^{Pi-T} \qquad \text{for } Pi > T \qquad \text{Eq.1}$$

$$\frac{r_{Pi}}{r_0} = 1 \qquad \text{for } Pi \le T$$

One of the most important assumptions in the model is that for plants with nematode density (*P*i) and without nematodes, the same total weight is obtained at a different age, the ratio rPi/r_0 is constant during the growing period. The variables r_0 and rPi are the growth rates for plants without nematodes and for plants at nematode density *P*i, respectively. The tolerance limit, *T*, is the maximum density below which the nematodes do not reduce plant weight. Parameters *z* and *k* are constants < 1. When nematodes cannot stop plant growth completely, even at very large nematode densities, growth still occurs: $rPi/r_0 = k$. The value of *k* is independent of nematode density and time after planting but varies between experiments. The fundamental model of the relation between nematode density (*P*i) and plant weight (*y*) was derived from cross sections orthogonal to the time axis through growth curves of plants for ranges of densities *P*i/*T* and different values of *k*. These cross sections are in close agreement with equation (2).

$$y = m + (1-m)\, z^{Pi-T} \cdot \qquad \text{for } Pi > T \qquad \text{Eq.2}$$

$$y = 1 \qquad \text{for } Pi \le T$$

Model parameters

Minimum yield

Minimum relative plant weight (*m*) is usually slightly larger than *k*. Parameter *z* is a constant < 1 with the same or a slightly lower value than in equation 1 and the parameter *T,* is the tolerance limit with the same value as in equation 1. Yield can be derived directly from plant weight (*y*), using relevant quality and financial data. Equation 2 was validated by fitting data from 36 plant-nematode combinations across 29 experiments describing the relation between *P*i of 14 tylenchid nematode species and the relative dry plant weight (*y*) of 27 plant species/cultivars several months after planting (Seinhorst, 1998). For crops prone to Seinhorst's second mechanism of growth reduction, equation 2 was expanded to a double exponential function (Seinhorst, 1986a). The phenomenon of "early senescence" of potato plants at tuber formation is not included in NemaDecide as it appeared to occur only in exceptional cases.

Tolerance

Tolerance of potato cultivars was quantified by expressing values of the tolerance limit *T* and the minimum yield *m*. Tolerance manifests at small nematode densities while *m,* at larger ones. Ranges of initial population densities (*P*i) are required to estimate either one of these parameters. The value of the tolerance limit *T* seems unaffected by differences in external conditions and could be estimated from both pot experiments in the glasshouse and field experiments. During glasshouse tests large enough pots must be used to prevent the plants becoming pot-bound resulting in biased estimates of *T*. The minimum yield *m*, is more sensitive to external conditions than *T*. Therefore, differences in tolerance between plant cultivars were established in one field experiment under the same conditions. In addition, enough values of *m,* were estimated in field experiments to establish a distribution function of *m*.

Population dynamics

For nematode species with one generation per year and become sedentary after host invasion, the fundamental population dynamic model (Seinhorst, 1967,1986a) in equation 3 is used:

$$Pf = M \cdot \left(1 - e^{-a \cdot \frac{Pi}{M}}\right) \qquad \text{Eq.3}$$

Initial nematode densities (*P*i, before planting) and final nematode densities (*P*f, after harvest) both are variables and expressed as nematode numbers (g dry soil)$^{-1}$. Where *a,* is the maximum rate of reproduction, *M* is the maximum population density (specific for each nematode/plant species combination). In this exponential model it is assumed that the size of the offspring is proportional to the part of the root system that is exploited for food and that *P*f = *a* * *P*i if *P*i → 0 and *P*f = *M* if *P*i → ∞**.** This model is based on the same principles as equation 1. Equation 3 only applies to the soil area containing roots (rooted zone). To predict population dynamics under field conditions, equation 3 was expanded in three ways.

1. The reduction in plant size caused by higher nematode densities than *T* results in a smaller, restricted, root system and therefore in a decreased food source and hence lower multiplication (Seinhorst, 1967). The compensation because of the decline of the root system has been added to equation 3.
2. The root system of the crop is not able to cover the complete volume of soil available in the field. Potatoes, grown in ridges, can root approximately 70% of the soil volume. In the other 30%, where no roots are available, natural population decline occurs (Seinhorst and Ouden, 1980). Therefore, equation 3 was expanded with a model for the population dynamics in the unrooted soil.
3. There is a reduction in numbers of eggs per parent nematode caused by intraspecific competition on highly resistant cultivars (Seinhorst, 1984).

Relative susceptibility (RS) and Relative virulence (Rvir)

To predict population dynamics of nematodes on resistant cultivars a stable measurement for resistance is needed. Therefore, the concept of RS was introduced. Relative susceptibility is defined as the ratio of the maximum multiplication rate (*a*)*,* of a nematode population on the resistant cultivar and on the susceptible reference cultivar $\mathrm{RS}_a = a_{resistant} / a_{susceptible}$ or the equivalent ratio of the maximum population density (*M*) on these cultivars $\mathrm{RS}_M = M_{resistant} / M_{susceptible}$ (Phillips, 1984; Seinhorst, 1984; Seinhorst & Oostrom, 1984). These ratios present two equal measures of RS, provided that the tested cultivar and the susceptible reference are grown under the same conditions in the same experiment and that both population dynamical models run parallel which was the case in more than 80% of the tested cultivars. When measuring RS one test reference population and two potato cultivars are used. A potato cultivar where RS must be estimated is tested against a susceptible refence cultivar, currently Desiree in the EU.

Relative virulence (RVir) compares the population dynamics of two nematode populations on one potato cultivar. It is defined as the ratio between the respective multiplication rates (*a*)-values of a virulent population and the reference *G. pallida* population as $\mathrm{Rvir}_a = a_{vir.pop} / a_{ref.pop}$ or the equivalent ratio of the maximum population density (*M*) $\mathrm{Rvir}_M = M_{vir.pop} / M_{ref.pop}$. The resulting population density in the field is then the result of RS and Rvir. **Note** : Whether or not a'_{pop}/a'_{ref} equals $M'_{pop}/M'_{ref\ pop}$ is unknown for the current virulent population (Emsland/Ampop). There is a great need to establish the population dynamical line of a breakthrough virulent population to estimate its true impact on potato cropping.

Parametrizing the parameters

The parameters used in the models are entered in NemaDecide as stochastic quantities, meaning that their distribution functions, mean and variance are known, unless they are true constants, such as the tolerance limit (*T*) . This information facilitates risk analysis, which is important for predictions on individual fields and for detection of sources of uncertainty that need further investigation. Field data collected by Seinhorst & Den Ouden; Schomaker , Been & Molendijk over more than forty years, were used to parameterize the growth model and population dynamic model. Since 1999, all new Dutch potato cultivars have been tested according to a new protocol to estimate RS. The protocol is safeguarded by the Plant Protection Service (NVWA) and this information is available for all new cultivars. Since 2010, the screening method, although slightly changed, is used in the whole of the EU. The current cultivar list in NemaDecide, now covering some 500+ cultivars, was established by screening for cultivars with possible resistance, based on the presence of resistance genes in their ancestors, and the RS estimation of all tested cultivars since 1999, except for the last couple of years. While the infrastructure of NemaDecide is in complete transition to Farmmaps, data on RS values of recently tested potato cultivars need to be added and updated to provide for state-of-the-art management scenarios.

Chemical control

Data collected by Seinhorst & Den Ouden; Schomaker, Been & Molendijk and the plant protection companies were used to estimate the effect of nematicides and nematistats on all relevant parameters of the population dynamics and yield loss equations. Nematicides kill a certain proportion of the nematodes in the tilth dependent on soil type and nematicide used. Their cost/benefit lies at extremely high nematode densities which a farmer should avoid using a good management system. Moreover, the environmental impact is so bad that using nematicides is almost forbidden in the EU. Treatment of plants with nematistats does not kill nematodes but delay nematode penetration into the roots and results in a certain fraction of the root system escaping nematode attack and, thus, remaining healthy. As a result, the minimum yield *m,* is increased by the fraction of the root system untouched by nematodes. Nematode penetration is postponed until the nematistats are no longer effective and the nematodes return to normal activity. Required concentrations of these nematicides to cause mortality of the nematode have been forbidden from the start of their use. Experiments on root feeding nematodes confirm that nematistats increase *m* and sometimes influence maximum yield (Y_{max}) but hardly affect the tolerance limit *T*. The tolerance limit *T* is only affected when nematodes die because of long lasting effects of nematistatics, which does not occur at the current concentrations. Dependent on dosage, nematistats also influence the parameters *a* and *M* of the population dynamic relation. Evidently most of the fumigants and non-fumigants are out of the market, the information, however, is kept in NemaDecide, as long as these chemicals are used within or outside the EU.

Required external data for decision making

1. Sampling results: The most important information required as input for NemaDecide is the initial population density (*P*i), in a farmer's field. Currently, about thirty different sampling methods for nematodes are in use, varying from rather extensive (200 cc. from 60 cores/ha) to intensive (13.9 kg from 331 cores/ha). Ten sampling methods for PCN are included in the system, together with their uncertainties. The Dutch General Inspection Service for Seed and Seed potatoes (NAK) was part of the NemaDecide consortium, and a webservice has been developed to enable extension officers to download the statutory sampling results from this sampling agency to NemaDecide when farmer permission is obtained.

2. Cultivar list: In the cultivar list, properties of potato cultivars are made available to farmers to enable selection of those cultivars which are most beneficial with respect to resistance, tolerance and other properties. **Fig. 2.** provides an overview of the presented information. Depending on the field distribution of the current virulent *G. pallida* population the list must be updated in the future with new RS values of new cultivars, currently under development.

Resistance: Now more than 500⁺ potato genotypes are present in the potato cultivar database. The RS to the pathotypes Pa2 and Pa3 of 50% of these cultivars was tested and entered to the cultivar list. This information exceeds the information about resistance as required by EPPO.

Tolerance: Tolerance is the ability of potato cultivars to prevent yield reduction by nematodes. This quality is expressed in parameter values *T* and *m* and in susceptibility to the second mechanism of growth reduction.

Cultivar properties: Except for information on nematodes, several other properties of the potato cultivars, such as susceptibility to other diseases or physical properties of potatoes including yield and skin colour, are entered into the database. A substantial amount of these data can be found in the "List of cultivars of field crops" which includes, the "recommended list of cultivars" and the "national list", annually provided by the Directorate of Agricultural Research. However, many data were provided by the breeders themselves, which were involved in the development of NemaDecide. All other breeders kindly provided the required information for NemaDecide after the project was started.

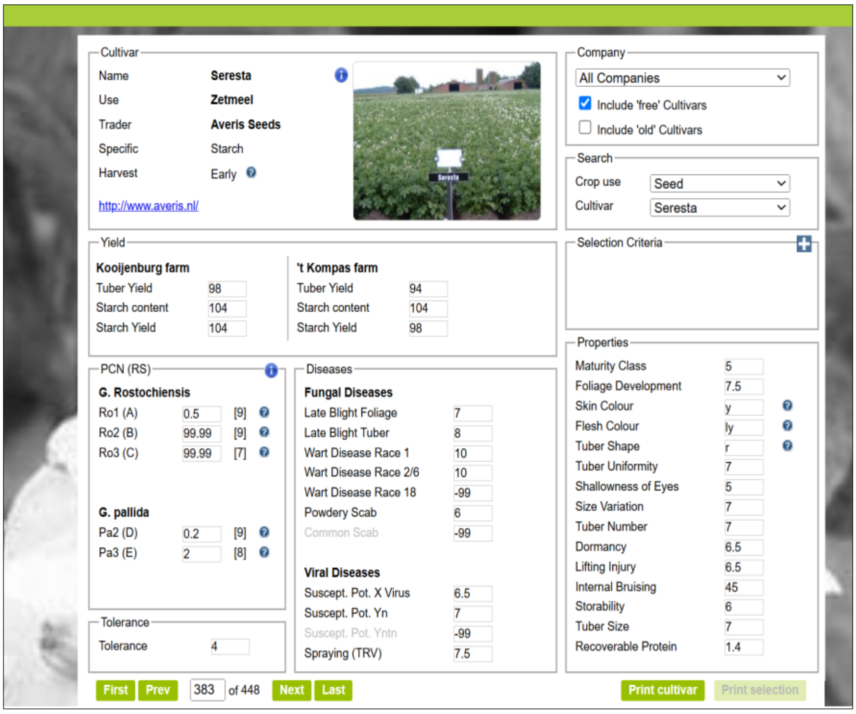

Fig. 2: Screenshot of the potato cultivar information window. To the left: Resistance to PCN pathotypes. Middle left: Fungal and viral diseases. Middle right: cultivar characteristics. Selections can be made on

Business house and three criteria. One can search for a cultivar or pinpoint only some preferred cultivars (to the right). If the checkbox is checked only selected cultivars will be available in the calculations.

3. Chemical control: To obtain objective decision rules for chemical control, the effectiveness of nematicides was expressed in terms of model parameters. The nematistatics were entered in full field/full dosage; full field/halve dosage and row application/quarter dosage.

4. **Financial Information:** Data on the cost of control measures (e.g. chemical control), the cost and benefits of sampling methods, were provided by the companies involved. Marketing information of farmers (expected maximum yields, market prices of products) can be added manually.

5. Legislation: Government rules concerning the management of quarantine nematodes are incorporated in the system. Official demarcation showing infested areas plus buffers according to legislation is added and implemented in the system.

6. Geographical data: In the first decade of the 21st century, the Dutch government implemented the EU rule to register every field in a digital database, which includes the field polygon, and the crop planted. This information is public through the BRP ("Basis Registratie Percelen", translated to "basic registration of a parcel"), providing a geo-referenced database containing the polygon of every field in The Netherlands (750.000 ha) and each crop grown on these fields for 17 years. In NemaDecide the geo-information from the government and the sampling results are combined, to integrate this information and to enable visualization. The sampling, Stripbuilder and NemaDecideGEO+ apps for their integrated functions mentioned above in farmmaps are shown below (**Fig. 3**)

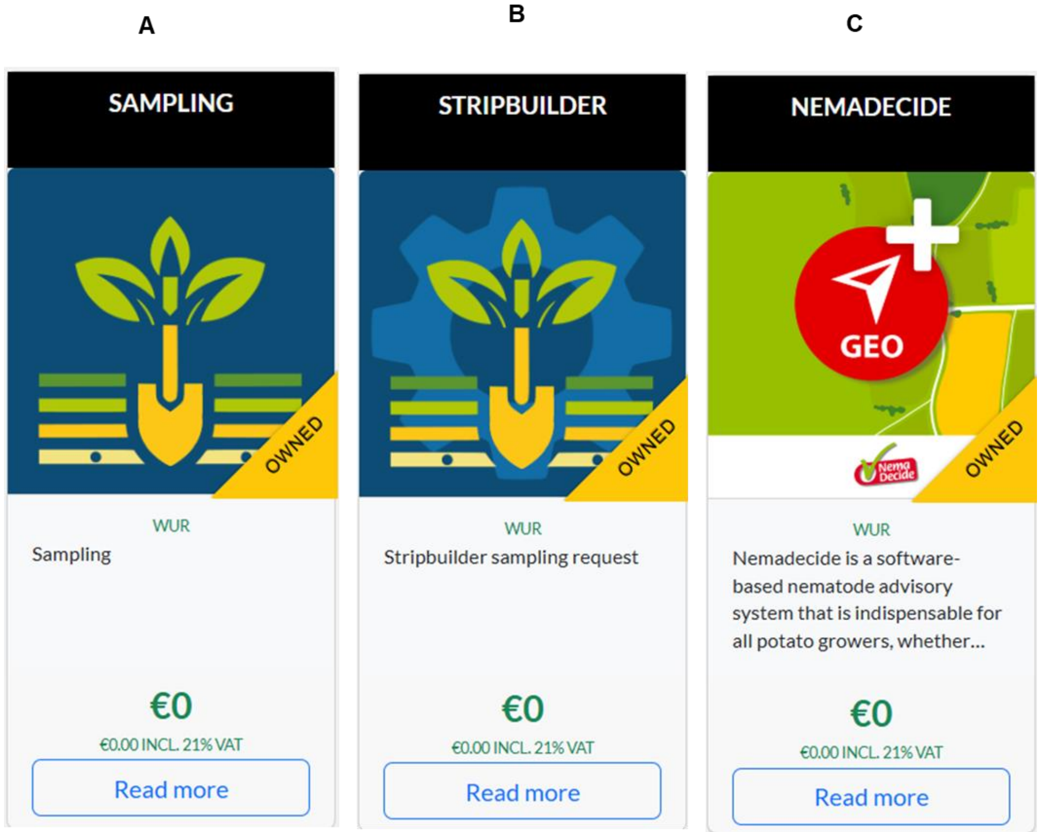

Fig 3. The sampling, the Stribuilder and NemaDdcide GEO+ apps as available in the store of Farmmaps.

Outline of the software

NemaDecide is split into two main simulation sections: one for infestation foci (**Fig. 4**) which applies to ware and seed potatoes and one for full field infestation (**Fig. 5**), mainly used for starch potatoes. Seed and ware potato growers use different soil sampling methods to starch potato growers. The EPPO method applies to ware and seed areas. Based on the sampling method requested by the farmers and the reconstruction of the sampling results, NemaDecide differentiates between the two simulation environments.

Infestation focus model

For seed and ware potatoes PCN sampling is carried out in strips. Ideally these strips are 300 meters long and 6 meters wide when dedicated methods are used (PN-intensive) or 300 m by 18 m when the EPPO method is applied. Strips are situated in the direction of cultivation and provide information about the area and intensity of the infestation. The software first analyses the sampling data and decides whether the infested strips found belong to one or more separate infestations. These infestations are then presented by a colour code showing different densities of the infestation (**Fig. 4**).

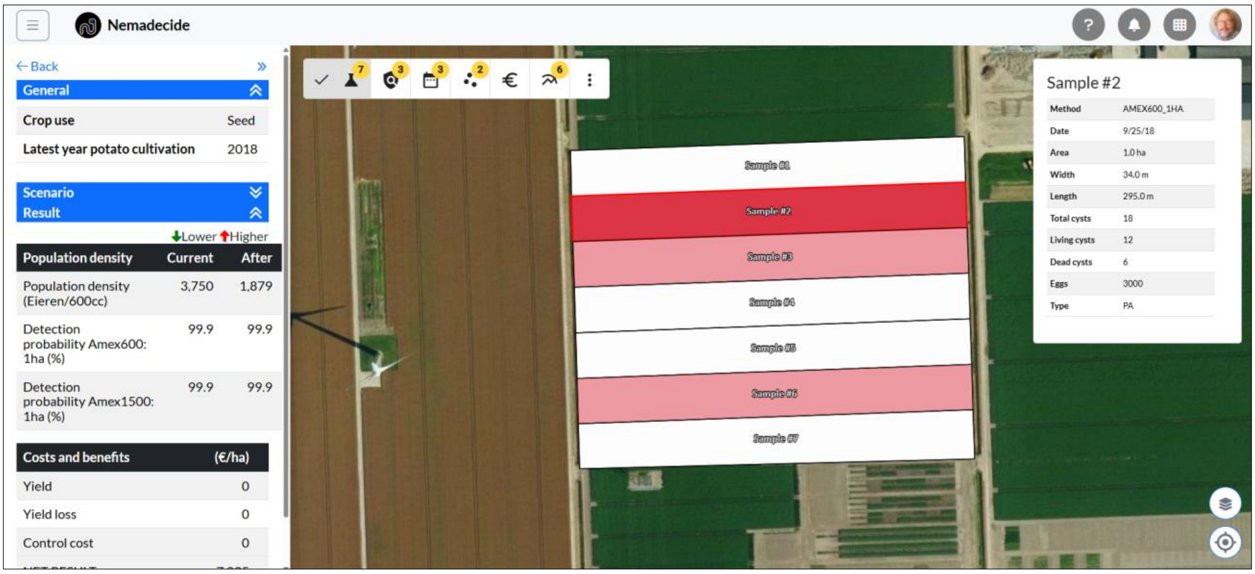

Fig. 4: Screenshot of the sampling result displayed on a satellite image, providing the location of the areas sampled and the individual samples results in the top right window when hovering over the sampled strip. Separate infestations are recognized and identified by colours for their density. Displayed here is that the software recognizes two infestation foci with *G. pallida*.

Next, an infestation can be selected for advice. The program then tries to recalculate the actual size and intensity of the selected infestation focus. If too many cysts are found in the infested strips the program issues a warning that reconstruction of an infestation foci is impossible and that a switch should be made to the full field simulation. If the data indicate the existence of an infestation focus, this size of the focus is estimated and displayed. The farmer now can choose various cropping scenarios, apply nematicides or nematistats, calculate the effect of volunteer plants and see the effect displayed both in the 3D graph and in the financial information frame.

To keep the farmer informed about the consequences of their actions for detection, the program consistently calculates the detection probability of the focus, using the EPPO sampling method and a chosen dedicated sampling method. Farmers can investigate which cropping scenario will lead towards small cost/benefit ratios and a low detection probability by statutory soil sampling methods. The long-term effect of resistant cultivars on the population density of PCN can be predicted within the limits that are determined by the uncertainties in the entered parameters and variables. Also long-term risks and financial losses caused by inadequate sampling methods can be calculated and visualised. Obviously, an initial population density estimated by an intensive sampling method is more precise than a *P*i-value estimated by an extensive sampling method. In NemaDecide the *P*i will be entered as a stochastic quantity, its uncertainty being determined by the sampling procedure used. This enables cost/benefit calculations on sampling methods and helps farmers to choose an optimal procedure for their purpose. To do this, we estimated the variance of all the sampling methods offered. In **Fig. 5** the difference in focus size is displayed when 2 cysts are detected with the EPPO sampling method and with the AMI100 sampling method. The AMI100 method was developed for seed potatoes in a 1:3 rotation. The AMI100 method detects significantly smaller infestation foci than would be detected by the EPPO method. These scenario studies clarify that investment of some extra money in accurate sampling methods has a positive long-term financial effect.

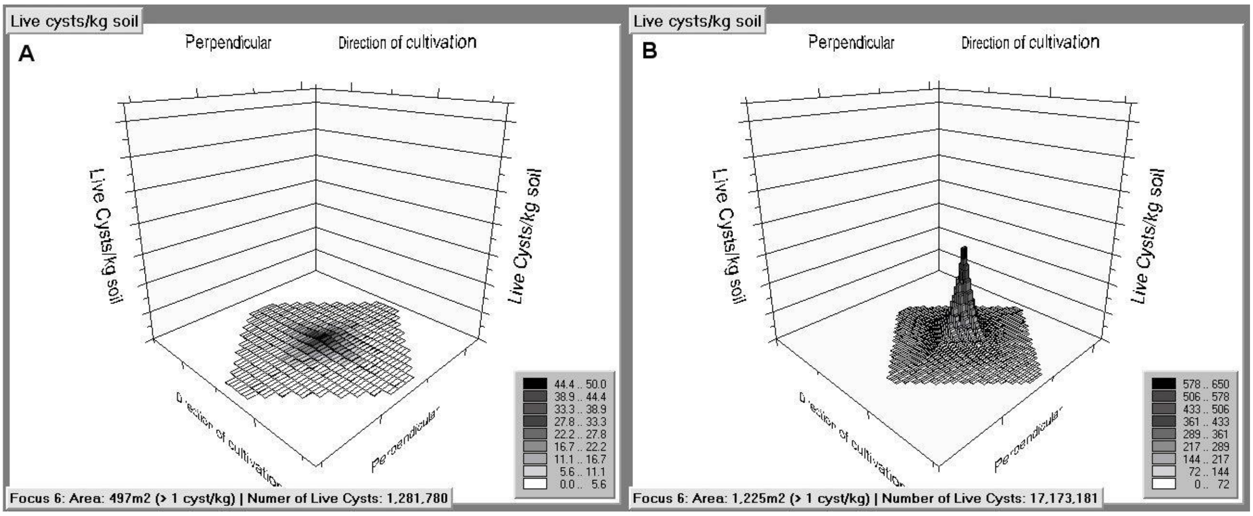

Fig. 5: Difference of the size of the infestation focus when sampled with the AMI100 **(A)** and the EPPO **(B)** sampling method. In both cases only 2 cysts are found. The former can detect infestation foci 1 to 2 susceptible potato crops earlier than the EPPO method.

Practical application of NemaDecide

Scenario 1: Full field model

In **Fig. 6.** a graph displays the average values of yield loss (bars) in the years when potatoes have been grown throughout the years in full field infestation. When the models are set to full stochasticity, the result of a calculation is a frequency distribution. Long-term effects of partial resistant cultivars and long-term risks and financial losses caused by inadequate sampling methods are calculated and visualised as in the focus model.

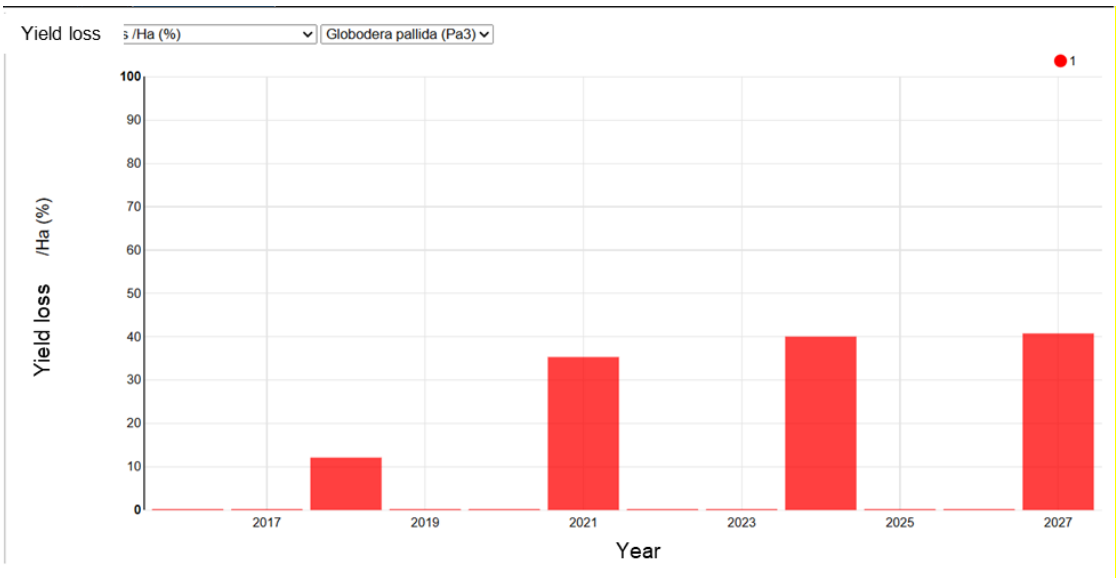

Fig. 6: Scenario 1: Screenshot of the expected average yield losses of a full field infestation in time. The susceptible potato cultivar 7four7 is used (RS = 100% for Pa3) grown in a 1:2 crop.

In the following cases illustrations on how NemaDecide is used to explain and discuss the general principles of managing PCN when used by extension officers and farmers is described. In these cases pathotype PCN, regardless of a virulent/avirulent population is presumed to be present with an initial population density of 5 eggs (g dry soil)$^{-1}$.

Scenario 2: The use of resistant cultivars in a 1:2 rotation (full field infestation)

Farmers often presume that it is impossible to control PCN without chemical control in short rotations. But when the RS of cultivars is 2%, as is the case with cv. Seresta, the yield reduction during the first year varies between 2.3 and 6.4%, with an average of 4.4%. In the next years, yield reduction is negligible if cultivars are grown with the same degree of resistance. After two years, the population density has declined below the tolerance limit (**Fig. 7**).

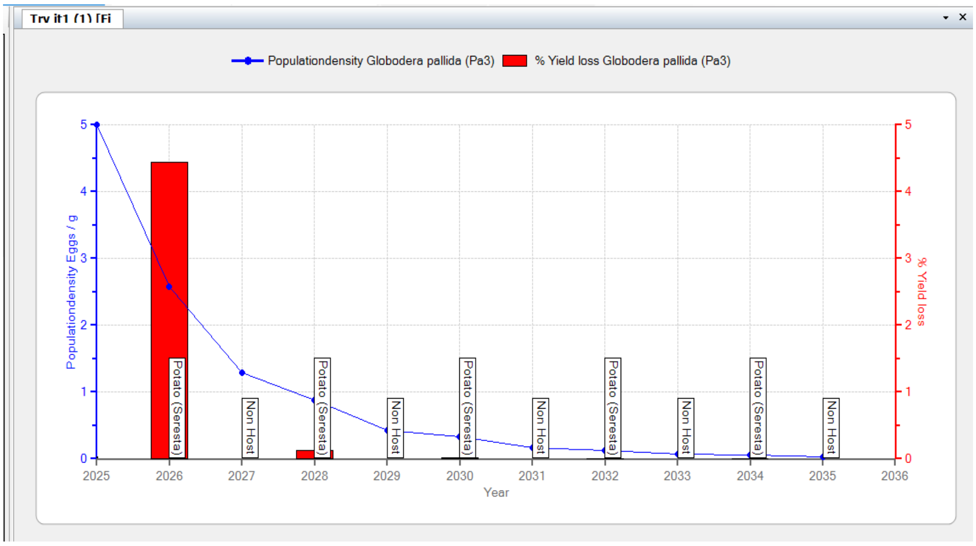

Fig. 7: Scenario 2. Screenshot of full field infestation (showing population change and yield loss) when, the resistant starch potato cultivar Seresta (RS = 2% for Pa3) is grown in a 1:2 crop during 5 rotations.

Scenario 3: The use of a cultivar with RS = 30% (formally susceptible according to the EU score)

Based on the status of a cultivar within EPPO regulations, farmers are made to believe that a cultivar like Santé (RS = 30%) is not useful to manage PCN. This is true in 1: 2 rotations, where yield reduction after some years stabilizes at an average of 24.4%, with a maximum of 50% (**Fig. 8A**). However, when Santé is cropped in a 1: 4 rotation, average yield loss is limited to 5.4 %, while the risk of a yield reduction of >10% is only 5.5% (**Fig. 8B**). In longer rotations with ware potatoes this degree of resistance causes acceptable yield losses. Presently 73 cultivars are available with a RS ≤ 30% or smaller for Pa3. NemaDecide visualizes the concept of RS resulting in an equilibrium population density based on the level of resistance and helps farmers to familiarize themselves with the relation between cropping frequency and partial resistance to choose levels of resistance that are sufficient for their specific needs.

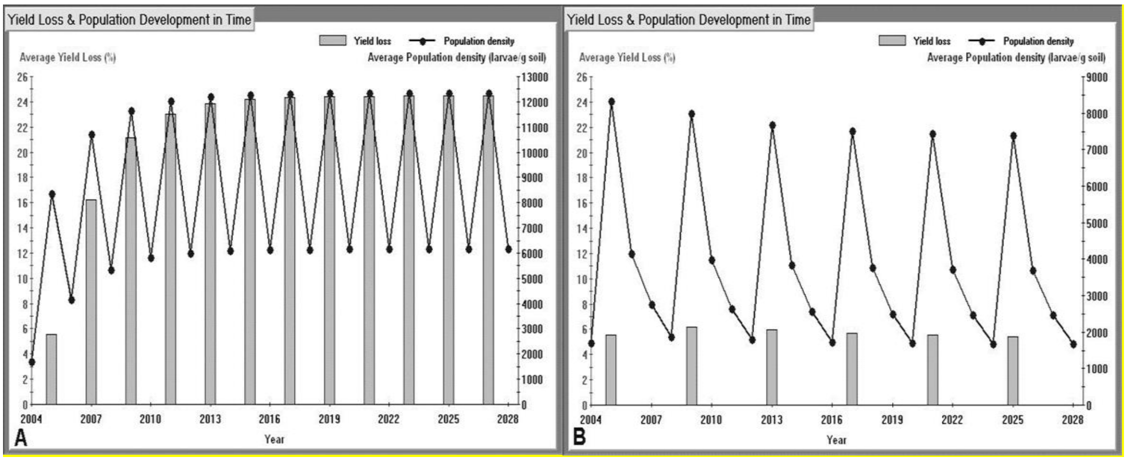

Fig. 8A and B: Scenario 3: Population density and percentage yield loss when cv. Santé (RS = 30% ; susceptible according to EPPO-standards) within a 1: 2 rotation **(A)** and in a 1:4 rotation (**B**). reduction in the number of cysts containing live eggs.

Scenario 4: Alternating susceptible and high resistant cultivars in 1:4 rotations. Another common misconception is that infestations can be managed by alternating susceptible and high resistant cultivars. As shown in the NemaDecide simulation, the population increase on the susceptible cultivar (Bintje) causes an average crop loss of 15% (95% quantile: 35%) in the next resistant crop (Innovator, RS =1%). Because of the population decline after the resistant cultivar, the susceptible cultivar can be grown almost without damage. Therefore, the resistant cultivar is believed to be less tolerant than the susceptible cultivar (**Fig. 9A**). This example helps the farmer to understand that resistance does not mean that the crops cannot suffer damage and that the use of fully susceptible cultivars should be avoided, even at small densities. Even the row application of a nematistat under optimal conditions, a sandy soil, cannot prevent an average yield loss of 7%. (**Fig. 9B**).

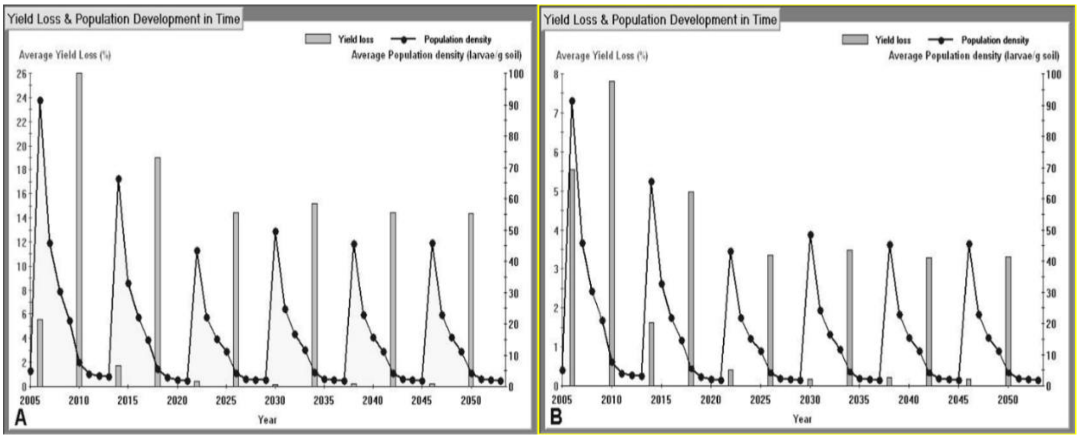

Fig. 9A and B: **Scenario 4**: Population density and average percentage yield loss in a 1:4 rotation where Bintje (RS =100%) and Innovator (RS= 1%) are alternated. **(A)** without and **(B)** with a row application of a nematostatic applied in the resistant cultivar.

Scenario 5: The effect of soil fumigation on the detection of PCN

In this example Bintje is grown every four years. We are dealing with an infestation focus. In one scenario a soil fumigation is applied in 2008 and in an alternative scenario there is no fumigation. It is shown that even though the soil fumigation results in a mortality rate of 70%, the detection probability is not reduced substantially. This is caused by the fact that after fumigation, there is hardly any reduction in the number of cysts containing live eggs. Of course, the infestation levels of numbers of eggs per unit of soil are decreased (**Fig. 10**).

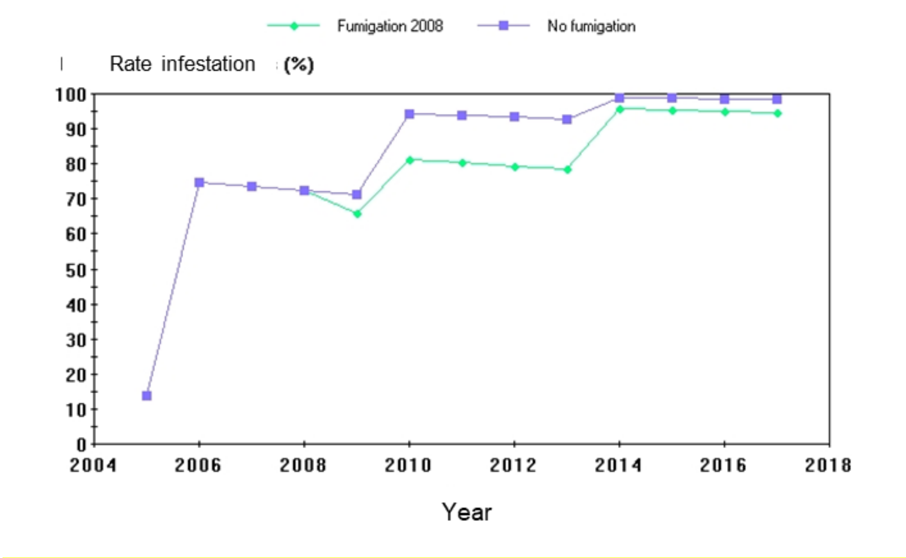

Fig. 10: Scenario 5: Soil fumigation in 2008 (↑) with a mortality rate of 70 % hardly decreases the detection probability of a focus.

Scenario 6: Detecting virulence

Assume that a virulent population is introduced into a field, this will increase the RS of the cultivars compared to the reference population. As the new virulent population is introduced to the old population, it takes some time, depending on the utilized cropping frequency before the new population takes over completely and the maximum population density (*M*) of this new population is reached. As a start NemaDecide will be able to spot these new virulent population when sample results in more cyst numbers than normally expected, when a recently resistant potato cultivar used. A virulence alert will be given. When the population dynamical parameters of the virulent population are known. NemaDecide can allow to do simulations for predicting the population development and forecast the yield loss. In **Fig. 11A** the comparison of different RS values of a virulent population on cv. Innovator and a susceptible cv. Bintje can be observed. The farmer then can select a potato variety bred for the new virulent population.

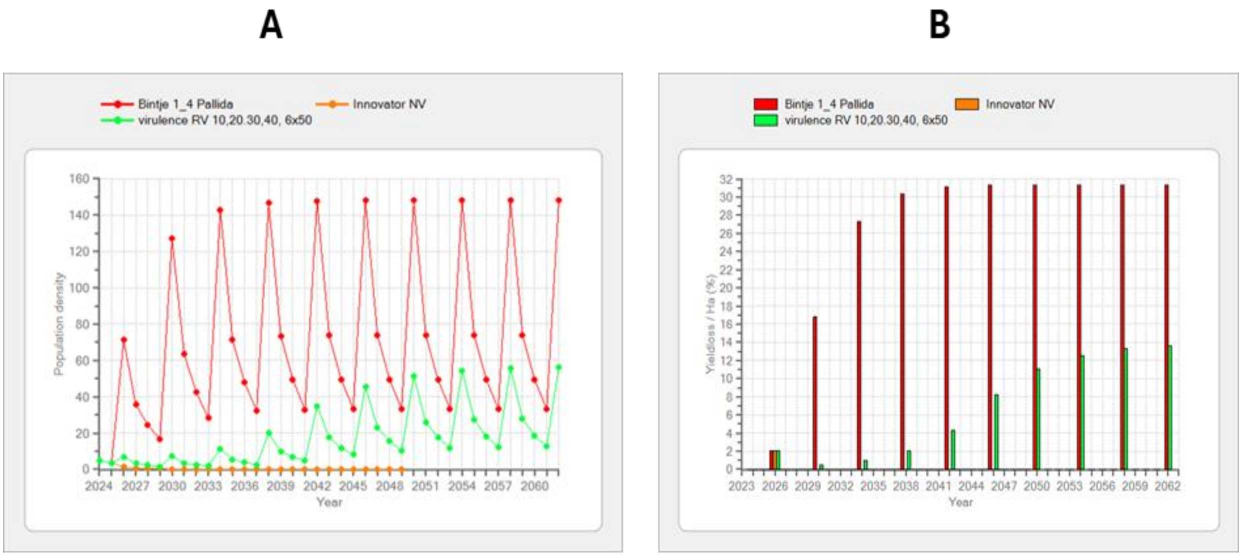

Fig. 11A and B: Scenario 6. Simulations for monitoring the population development of a virulent population on the resistant cv. Innovator if the RS might increase from 2% to about 50% and how the population development (**A**) and damage (**B**) looks like in a span of 1:4 rotations.

Research carried out

The infrastructure required to run NemaDecide and the assisting software on Farmmaps was supported while at the same time the research began to investigate if the existing soil sampling method for the starch potato area. The TBM sampling method is usable as a warning system for virulence occurrence.

Based on the performance of the sampling method it was established that when the virulent population begins to take over the 'normal' population, population density rise in such a level that virulent populations can be distinguished from 'normal' populations. For this a simulation model was developed taking cropping frequency and ultimate RS of the virulent population into account and using the population dynamics models to establish a timeline of population change. The first result was that an increase of a RS of 2%, like Seresta to 10% under a virulent population poses no problems when looking at yield losses, especially when still in the form of an infestation foci. The TBM method will provide for detection of a virulent population starting at RS = 15% for foci of the virulent population. In **Fig. 12A**. The population densities then are significantly higher. An identical approach has been used for full field infestations of the virulent population. Here the method works even better in **Fig. 12B**. For Example at RS = 2% the distribution of possible densities lies between 0 and 250 while at RS = 15% the distribution starts at 400 up to 2000 eggs (g dry soil)$^{-1}$. Any density found above 400 eggs (g dry soil)$^{-1}$ indicates a virulent population.

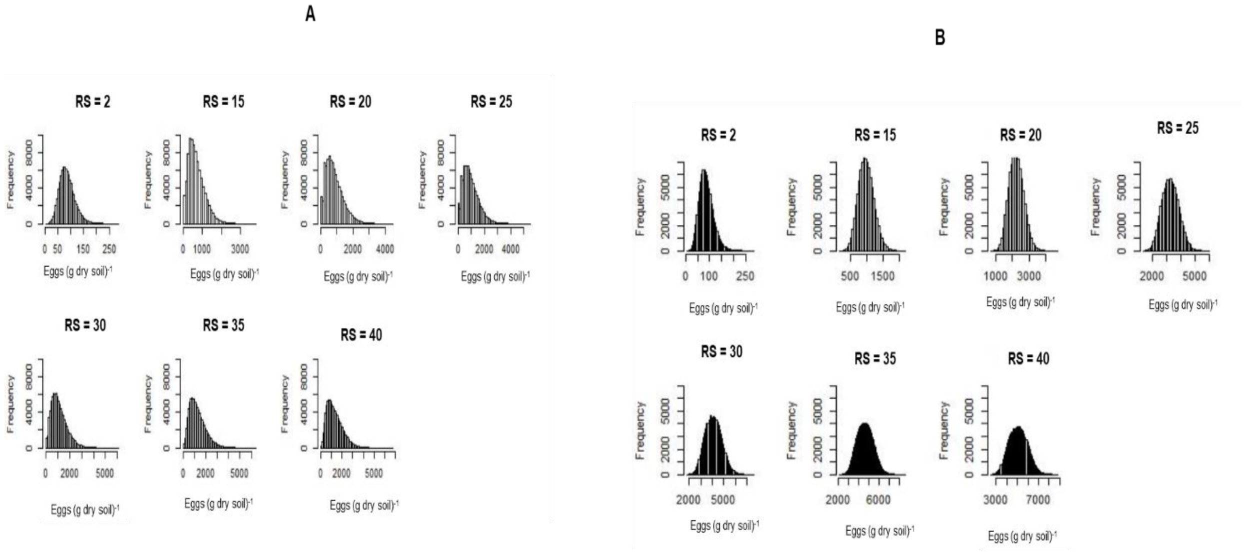

Fig. 12A and B. Frequency distribution of population densities found with the TBM soil sampling method for different RS values (due to increase of virulence) after 10 years of cropping. **A**). infestation focus **B**). full field infestation.

Research is now focussed on whether detection can occur earlier in time and what kind of enhancement of sampling method is required to establish this. Once this is complete, other sampling methods and the statutory soil sampling methods will be researched**.**

Suppression of emerging virulence

Next to detection and early warning of virulence comes management. Searching novel sources of resistance from wild potato genotypes is a long-standing option, even though it takes time before releasing a cultivar having the resistance and other desirable agronomic features. Prevention or suppression of the frequency of the alleles that govern virulence is an essential tool. As part of management of the newly emerging virulent populations of PCN, a comprehensive work was done in France (by INRAE and FN3PT) about masculinizing resistance combined with adjusting rotation schemes and biocontrol strategies. The work resulted in a demo-genetic model (i.e. tracking both nematode population densities and virulence allele frequencies) considering the population genetic features of PCN: diploid, sexual reproduction, recessiveness of the virulent allele and no virulence cost. The model showed that using masculinizing resistance, avirulence allele could persist in males and thus in the population. The model can predict the number of years needed to keep populations below the damage threshold and showed that combining masculinizing resistance, rotation, and biocontrol may achieve durable suppression of *G. pallida* in a reasonable time frame. This work is supported by an online interactive interface allowing users (i.e., growers, plant health authorities, researchers) to test their own control combinations (Tankam-Chedjou *et al*., 2024). It be further explored within the period of the NemEmerge project as a tool for farmers to neutralize the emergence of virulent population.

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