REGISTRATION FORM (BASIC DATA)

1a. Details of applicant

In case the applicant is a professor, it is assumed that he/she is also the promoter. In case the applicant is an associate or assistant professor (UHD or UD) the promoter must be mentioned under 1b.

Name, first name, title(s): Prof. Dr. H. J. (Harro) Bouwmeester
Male
Institution: Wageningen University
Department: Laboratory of Plant Physiology
Position: **X** Professor O Associate professor (UHD) O Assistant professor (UD)
Permanent position: **X** Yes O No, end date contract:

1c. Proposed PhD candidate

Name, first name, title(s): Boerefijn, Joël, BSc (Male)

2. Title of research proposal

To defend or to grow when phosphate is low.
The trade-off between herbivore-induced defence and phosphate limited growth in *Arabidopsis thaliana*.

3. Summary of research proposal

(scientific summary in English, max 250 words)

In nature plants are subjected to simultaneous abiotic and biotic stresses. To cope with these stresses plants need to strategically prioritise their resources to the organs with the highest need. When plants are subjected to combined abiotic and biotic stresses, multiple organs are in direct competition for resources. This leads to the allocation of resources to the organs with the highest nutritional demand. In this research proposal I will investigate the response of *Arabidopsis thaliana* to phosphate-limitation (abiotic stress) and spider-mite (biotic stress) infestation. Where do plants invest their resources when under biotic and abiotic stress simultaneously: defend or grow?
In this project I will first further elucidate how Arabidopsis responds to spider-mites with respect to investments in secondary metabolism and increased local sink strength. In Parallel I will also investigate how Arabidopsis responds to low phosphate with an local sink strength increase and a change in root architecture. I will combine these two stresses and investigate further how the strength of the different identified sinks changes during the combined stress treatment. These findings will be used to identify which (regulatory) genes and biosynthetic pathways are involved in the plants ability to determine the choice between growth and defence. This knowledge will help breeders and agronomists to select crops that prioritize right and to find agricultural measures that optimize how plants cope with multiple simultaneous stresses.

4. Main field of research

(state code and field of research; http://www.nwo.nl/financiering/nwo-disciplinecodes):
Biology; 20.50.00 Botany

5. Summary for the general public

(please provide in 100 words a title and summary for the general public, preferably in Dutch)

Planten zijn in de natuur vaak blootgesteld aan verschillende soorten stress. Deze stress kan bestaan uit de vraat van insecten maar ook een tekort aan bepaalde voedingstoffen (bijvoorbeeld fosfor). Om met zulke stressen om te gaan investeren planten tijdens vraat in de productie van stoffen die schadelijk zijn voor de insecten of bij fosfortekort in meer wortels. Deze reacties vergen van de plant een behoorlijke investering. Zodra planten blootgesteld worden aan meerdere stressen tegelijk moeten ze kiezen op welke stress ze zullen reageren. In dit project wil ik erachter komen hoe planten deze keus maken.

DESCRIPTION OF THE PROPOSED RESEARCH

6. Description of the proposed research

Introduction

Plants, unlike animals, cannot move away from stress factors. Instead they respond to stress by dynamically changing their physiological responses, such as changes in gene expression, biosynthetic pathways or resource allocation patterns. Examples of this are; the production of defence compounds in parts of the plant that are under attack by herbivorous pests (biotic stress) [1], and the production of more roots to create a better foraging potential when nutrients are limiting (abiotic stress) [2]. These responses occur in response to a single stress (biotic or abiotic), however when plants grow in a natural environment combined or sequential abiotic and biotic stresses are common [3,4]. With the increased societal and legislative constraints on the use of pesticides and fertilizers, these situations will also increasingly occur in agriculture. In order to cope with these multiple stresses plants need to invest their resources strategically in the different organs that each have different nutrient requirements [5]. This raises the question, which is also the essence of this proposal, where do plants invest their resources when they are under simultaneous biotic and abiotic stress: defend or grow? Both from an ecological and agriculture perspective it is important to obtain a more fundamental understanding of how plants can grow efficiently whilst dealing with defence under low nutrient conditions.

Arabidopsis thaliana will be used as the model for this research because of its short generation time, extensive collection of mutants, availability of molecular tools*,* and because it is a member of the extensive Brassicaceae family which also contains numerous economically relevant crops, such as broccoli, cabbage and rapeseed. As the abiotic stress factor I will study phosphate (Pi) limitation. Pi is an essential nutrient for plant growth and is involved in metabolism, membranes, nucleic acids, energy storage and protein regulation [6] . In most soils Pi accessibility is the principle limiting factor for plant growth. In most developed countries the use of non-renewable fertilizers helps to overcome, or delay, this problem. However, this temporary solution is expensive and finite [7]. As a biotic stress spider-mites (*Tertanychus urticae*) will be used. Spider-mites are cell-puncturing herbivores that feed on many (>200) plant species [8] including *A. thaliana* [9]. Because of its wide range of hosts, spider mites are economically a very important pest [8].

Background

To elucidate the trade-off between defence and root growth I will look at this problem from a source-sink perspective: how do plants change the source-sink balance between organs? The source organs of a plant are those organs that produces more carbohydrates than they use, this is usually represented by mature leaves, but may also include storage organs such as stems and roots [10] . Sink organs are those organs that use more resources than they produce, and they hence attract carbohydrates, for example young leaves and roots. Sites of carbohydrate production and storage, the source, can be relative far away from the sink. Plants therefore need a system to transport carbohydrates over long distances [11]. This transport of carbohydrates, which is mainly in the form of sucrose [12,13], is facilitated by the phloem.

A common response of plants to biotic stress is the production of defence compounds such as terpenes [14], glucosinolates [15] and phenolics [16], which is a costly process in terms of carbon use. Plants have two kinds of defence. In constitutive defence, certain defence compounds are always present independent of whether the plant is under attack or not. This type of defence is costly [17] especially when not required. In contrast, induced defences are considered as cost saving as the resources required for defence are only temporary. When parts of the plant are under attack by insects or other arthropod herbivores such as spider-mites, the local production of carbon intensive defence compounds increases [9] and this increases the sink strength of these organs. Ferrieri and colleagues [18] showed that there is a rapid relocation of carbohydrates from leaves that are not treated with methyl jasmonate (MeJA), a hormone up-regulated during herbivory, to sink leaves that are treated with MeJA. In those leaves the carbohydrates where incorporated into cinnamic acid, which is a precursor of various defence compounds. In developing populus leaves under attack by insect herbivores or treated with JA the import of carbohydrates from parts of the plant that were not treated/under attack increased by 400%. The relocated carbohydrates were used for the production of several defence compounds such as polyphenols [19,20]. Insect herbivory/JA treatment can also cause plants to retract their valuable nutrients from the infected sites and store them in parts of the plant that are not under attack. Those stored nutrients can then later be invested into the new growth of vegetative tissues. This strategy increases the plants ability to tolerate herbivory [21,22,23]. This strategy, however, is mainly used by perennials that can easily store nutrients, and not by annual plants such as *A. thaliana* that do not have long term storage capacity.

Alteration in the pattern of nutrient allocation does not only change during biotic stress, but also in plants that are under abiotic stress. Low-Pi availability is first signalled in the leaves. Sugars produced in photosynthesis serve as building blocks for cell membranes, amino-acids, proteins etc., and Pi is an important component in these processes. When Pi is limited, sugars can no longer be converted into these cellular components. As a result sugars are building up, which are then transported via the phloem to the roots [24]. This will result in an increase in the root:shoot (R:S) ratio [25,2], enhanced root proliferation and increased secretion of organic acids by the roots [26,27]. Transport of carbohydrates (sugars) from the leaves to the roots makes the roots a stronger sink than the leaves during Pi deficiency [26,28]. Reduction of nutrient availability may also lead to an enhanced investment in defence compounds [29,30]. The rationale is that plants growing under nutrient limited conditions cannot afford to lose large parts of their biomass to herbivory, and to avoid this they invest in increased production of defence compounds. At the same this serves as a mechanism to store excess carbon and other nutrients. Troufflard and colleagues [31], for example, showed that under potassium deficiency *A. thaliana* plants have increased production of oxylipins and glucosinolates to enhance their defence potential against herbivory and create a reversible storage of excess nitrogen and sulphur.

When plants are exposed to several types of stresses simultaneously, they need to decide which "defence" process to prioritise. Two important pathways involved in regulating the choice between growth and defence are jasmonic acid (JA) and gibberellin (GA). On the one hand, JA induces defence pathways against insects, and concomitantly supresses growth, whilst on the other hand GA promotes multiple plant growth responses [32]. GA promotes growth by stimulating the degradation of growth-repressing DELLA proteins [33]. JA is induced by herbivory and stimulates defence by promoting the production of a specific set of defence genes, such as *VSP2* while at the same time inhibiting growth by delaying GA-mediated DELLA protein degradation. These two pathways antagonistically interact at many levels. For example, in Arabidopsis, transcription factor MYC2 binds to the promoter of two sesquiterpene (volatile defence compound) synthase genes, *TPS21* and *TPS11,* and activates their expression. This expression is further promoted by JA/GA but is negatively affected by DELLA [43]. DELLA mutants on the other hand are less sensitive to JA-mediated growth inhibition [35]. Pi starvation responses in both shoot and root are repressed after exogenous application of GA as well as in a quadruple DELLA mutant [36]. DELLA proteins act also as positive regulators in the sucrose signalling pathway controlling anthocyanin biosynthesis and growth [37]. This makes DELLAs a key regulator in each aspect of the integration between defence and growth. Yet, is this pathway the key to re-allocation of resources to defending organs when carbon could be used elsewhere to alleviate a shortage of nutrients? And if so what are the critical components involved at the molecular level? These are the question we will answer in this project.

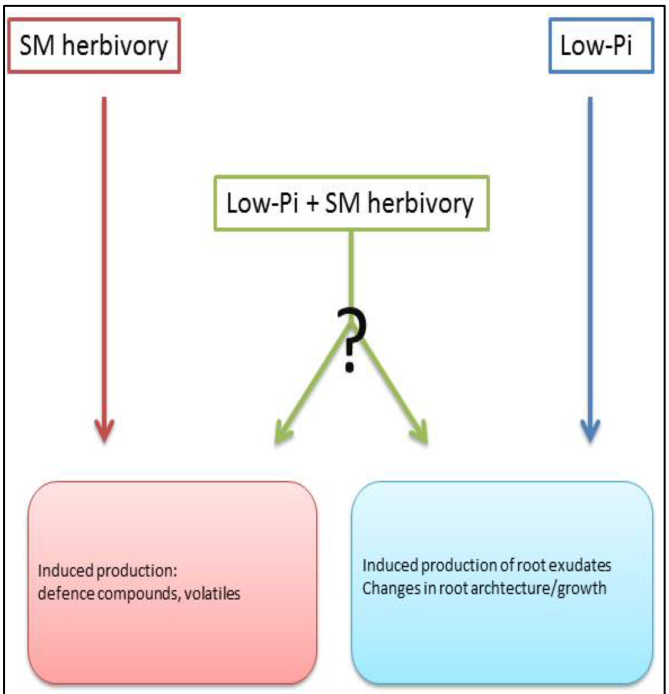

Figure 1: different stress factors are triggering different responses

Approach

In this project I will investigate how Arabidopsis responds to biotic stress with respect to investments in secondary metabolism and increased local sink strength. Parallel to this I will also further elucidate how Arabidopsis responds to low phosphate through alteration of local sink strength and changes in root architecture. Finally I will combine these two stresses and investigate how the strength of the different identified sinks changes during the combined stress. Using transcription analysis in wild-type Arabidopsis plants subjected to both stresses will identify genes that are involved in the mechanistic regulation of trade-off between these two stresses. in parallel an experiments will be performed with plants mutated in known defence and growth pathways. Finally, the involvement of candidate genes will be confirmed using the natural variation for defence and P-starvation response present in Arabidopsis.

Work-package 1: characterization of spider mite- and low Pi-induced distribution of carbohydrates
Aims: a) To identify the localisation and temporal dynamics of spider mite-(SM)/low-Pi induced sink strength in key tissues. b) To quantify carbohydrate distribution over the different sinks during SM infestation / low-Pi and combined stresses. c) To measure the impact of SM infestation/Low-Pi and both stresses combined on defence (secondary metabolism), hormone signalling, and root properties.
1a) Wild-type (WT) Col-0 Arabidopsis plants and different reporter lines (starvation:LUC; sucrose transporter:LUC (SUC2:LUC); already available at Plant Physiology, WU) will be grown on hydroponics under controlled conditions (light, temperature, nutrients etc.). When the plants have reached the ten rosette leaf-stage, different combinations of stresses will be applied (SM/JA, low-Pi and both stresses combined). To induce a defence response I will establish a working setup with JA (JA is a reasonable mimic of SM) first. After this setup is established I will introduce SM to induce defence responses. At different time-points, leaves, stems and roots of the reporter lines will be analysed for promotor activity. In this way the spatial distribution and time-points of the sinks created by different stresses will be identified.
1b) The distribution of carbohydrates over the identified sinks (obtained from 1a) will be analyzed in Col-0 wild-type (WT), *pgm-1* (deficient in starch-synthesis), and *atsweet11,12* (sucrose efflux transporter) mutants. Plants will be subjected to the different stresses (and combinations). After certain time point(s) (obtained from 1a), root and shoot fresh and dry weight, primary metabolites (sugars, starch, amino acids, organic acids), stress related hormones (JA, SA, ABA) and direct (e.g. glucosinolates) and indirect (volatile metabolites) defence compounds in leaves, stems and roots will be analysed. For these measurements LC-MS (Liquid Chromatography – Mass Spectrometry) and GC- MS (Gas Chromatography – Mass Spectrometry) will be used, respectively.
1c) To analyse changes in root architecture and root growth *Arabidopsis* plants (WT, mutants from 1b) will be both grown in hydroponics and on 12*12 cm^2 square plates. Plants grown on hydroponics will be treated with different stress regimes to induce associated responses, when the ten leaf stage is reached (similar to 1a), these plants will be used to analyse amounts and composition of root exudates produced in the roots (protocols available at PPH, WUR). Plants grown on square plates filled with an agar based nutrient solution (with similar amount of nutrients compared to hydroponics) will be used to measure different root parameters. Because plants growing on plates are spatially restricted in their growth, different stresses will be applied not at the 10 leaf stage (as in hydroponics) but at 6 days after germination (DAG 6). Plates will be scanned with a flatbed scanner at DAG 8,10 and 12. Pictures will be analysed with the software program EZ-Rhizo [38]. With this program up to 17 different root parameters will be measured (e.g. main root length, number of laterals, apical zone etc.). Using the plate system, and comparing the results with what happens in vivo (hydroponics) will give us more detailed information about the changes in the RSA induced by different stresses. I have previous experience with the experimental set up and imaging systems used in this objective. The RSA analysis will be executed in collaboration with Dr. Anna Amtmann (Glasgow University, UK), where I have previously completed an internship.

Work-package 2: Identification of genes involved in the prioritization of sinks
Aims: To identify genes involved in the prioritization of sinks a multi-disciplinary approach will be used; first, I will use RNA-Sequencing transcriptome profiling on roots and shoots of plants grown under the individual and combined stresses to compare the transcript profiles of plants. Using transcriptome data I will identify those transcripts differentially induced or suppressed in response to the different stresses (WP2a); secondly, I will use a series of known signalling/growth mutants to identify the potential trade-off between combined stress, measuring plant and herbivore fitness traits (WP3). Finally, combining RNA-Seq. data and information from the mutant screen I will select a list of candidate genes for further functional and molecular elucidation of the molecular network underlying the trade-off between defence and root growth in WP4.

2a) The effect of the different stresses (SM, low-Pi and combined) on the transcriptome will be assessed. Leave tissue/ root tissue (or other tissues depending on the outcome of work-package 1a), will be harvested from Col-0 WT plants grown under the different stresses (this material is generated in 1b). The time-points of choice will depend on the outcome of work-package 1a, but most likely early, middle and late time points. For transcriptional analysis RNA-Seq. will be used. Total RNA of different plant parts will be isolated and enriched for mRNA using oligo(dT) magnetic beads. A cDNA library will be synthesized and sequenced by Illumina HiSeq™ 2000. Data analysis will be performed using the transcriptome data-analysis pipeline developed by the department of Biometris (WUR) which is used in PPH. After data assessment, gene expression levels will be calculated according to the RKPM method and after normalization and FDR, differentially expressed genes will be further analysed using GeneMath software.
2b) The dataset obtained in 2a will help to dissect the molecular pathways underlying the prioritization of sinks. Key genes differentially expressed under the different stresses and plant organs will be validated using qPCR on independently generated plant material. RNA-Seq provides the dynamic range and sensitivity required to identify those genes expressed to low levels, e.g. transcription factors, which will be primary candidates in regulating the trade-off between growth and defence. Candidate genes confirmed to be induced (or repressed) will be further characterized in WP4.

Work-package 3: Are known defence/growth pathway genes involved the prioritization of sinks.
Aims: much is known about the mechanisms plants are activating during abiotic and biotic stress alleviation, yet the key regulation of the choice between the two is not. Here we will take advantage of the rich knowledge available to: a) characterize the effects of known defence/ growth pathways on the strength of the sinks and their prioritization, and b) to integrate the effect of candidate genes from 2b in the system.
3a) Based on the results obtained from 1b we will establish the relevant parameters to define the relocation of resources and the defence effectiveness of established mutants in key pathways related to the question at hand. As a preliminary list we will look at: Defence response related mutants with constitutive expression (or parts of) of jasmonic acid (*sev1*), ethylene (*cet1*), deficient in (or parts of) jasmonic acid signalling (*coi1, jar*), ethylene (*ein2*), abscisic acid (*aba2-1*) and salicylic acid induction (*sid*); growth mutants hypersensitive to auxin and cytokine (*bud*) and DELLA (*ga1-3 gai-t6 rga-t2 rgl1-1 rgl2-1*); important transcription factors activating secondary metabolites such as MYC2,3 & 4 (*myc)*; mutants related to the resource re-allocation during low-Pi The results of these experiments will confirm whether certain pathways/genes are involved in sink strength increase of certain organs during the induced stresses and whether they play a role in prioritization. This first analysis will help us to anchor and filter out the results from the RNA-seq and pinpoint the key integrating factors in the resource allocation.
3b) using the results obtained from the WP 1,2 and 3a we will construct a multi-level network. This network will be queried for the key nodes in the re-allocation of resources during the dual stress scenario. The methodology developed by Ronny Joosen (Identifying Genotype-by-Environment Interactions in the Metabolism of Germinating Arabidopsis Seeds Using Generalized Genetical Genomics 2013). Based on these results we will extend our list of candidates genes from 3a.

Work-package 4: Confirm and characterize candidate genes and pathways in *A. thaliana*.
Aim: Confirmation of the importance of the identified genes in WP2 and 3 in *A. thaliana*
Candidate genes from 2b that were confirmed using qRT-PCR in independent experiments will be cloned. Their contribution to sink prioritization under SM and low-Pi conditions will be characterized using both knock-out (KO) mutants from publicly available sources and over-expressing (OE) transgenic lines in different backgrounds (e.g. crossing into one or more of the mutants described in 3a).

Work-package 5: Confirm (characterize) candidate genes and pathways using natural variation in *A. thaliana*.
Aim: In this WP we will confirm the characterized genes from WP4 using natural variation.
The *A. thaliana* HapMap collection including 360 accessions has been screened for various traits including thrips (*Frankliniella occidentalis*) infestation and low Pi availability (Laboratories of Entomology, Genetics and Plant Physiology (all WUR)). Extreme responding ecotypes (very susceptible and/or resistant to the stresses) will be selected. As thrips like spider mites, are cell-puncturing herbivores we expect that ecotypes resistant or susceptible to thrips will have a comparable response to spider mites. Indeed, a number of ecotypes highly resistant to thrips were also found to have high resistance to spider mites and vice versa in a much smaller

groups of ecotypes ([9] and Kappers unpublished data). Selected ecotypes will be subjected to SM and/or low Pi and will be analysed for primary and secondary metabolites and root architecture as described above. Genes of interest from WP2 and 3 will be analysed using qPCR and the most appropriate methods for metabolite re-allocation from 1b.
Taken together we will dissect the decision making process a plant is going through when facing a decision between responding to low-Pi and defending it leaves from SM. The expected outcome will be clear and novel targets for breeding plant with high level of defence in unfavourable environments and a better understanding of the multi-stress factor integration at the molecular level.

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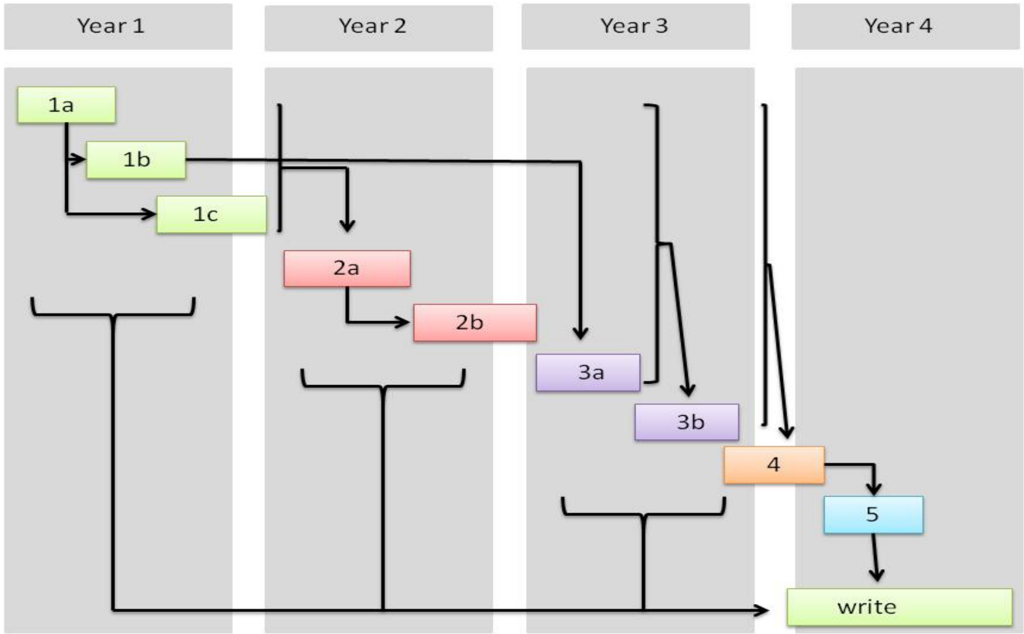
7. Timetable of the project

Give a practical timetable over the grant period, max. 0.5 page.



The proposed program will be executed in four years (Figure above) resulting in the writing of a PhD dissertation. The five independent chapters are interconnected. **Year 1** is dedicated to the characterization of spider mite- and low Pi-induced distribution of carbohydrates. This will basically mean that we setup a working system where time and place of stress induced sinks are known. In **Year 2** I will identify new genes by using transcriptome data from RNA-sequencing. in **Year 3** I will use the abundant knowledge about pathways activated during abiotic and biotic stress to identify key regulation pathways of the choice between those two. In **Year 3** identified genes/pathways will be further characterised. In **Year 4** we will confirm the characterized genes from year 3 using natural variation in *A. thaliana*.

8. Economic and / or societal relevance

Describe the relevance of the results and/or insights from the research for and the contribution to solving societal and economic issues relevant to the topsector Horticulture and Starting Materials.

With an ever growing world population and the shrinkage of fertile arable lands [39], we are facing an enormous challenge to feed the human population in the near future. To maintain a high yield, excessive use of pesticides and fertilizers are required to keep biotic and abiotic stress under control. However, rock phosphate reserves are declining fast and it is estimated that in the next 50 to 100 years these reserves will be completely depleted [40,41]. This problem is already recognized by the fertilizer industry, that confirms that not only the quantity but also the quality of these reserves are shrinking. Associated with this the costs of extraction, processing and shipping are increasing [42,41]. The excessive use of fertilizers has also led to a large run-off of nutrients into ground water and surface water, which has led in some cases to the growth of toxic algae blooms [43]. Intensive use of pesticides, in combination with the rapid generation time of harmful arthropod herbivores, has led to an increasing resistance to pesticides, also in many spider mite populations around the world. A lot of knowledge is available about the response of plants to these stresses separately. The novelty in this project is that I will gain insight into what exactly occurs in naturally realistic situations, when plants are exposed to both biotic and abiotic stresses simultaneously and how prioritization of sinks is regulated by plants. This knowledge will help breeders and agronomists to select crops that prioritize right and to find agricultural measures that optimize how plants cope with multiple simultaneous stresses.

9. Composition of the consortium and in-kind matching

Indicate the private and/or public partners involved in the project and the in-kind matching that is required.

We have not been able to identify a public partner yet but – considering the huge importance of insect resistance and phosphate availability - are confident that we will be able to do so if the project is funded.

FINANCIAL DETAILS

10. Budget

Please use the table below for the description of the personnel and material resources required for the project. The maximum budget that can be requested from NWO is € 231,250, including cash matching the total cash budget is k€ 250. In-kind contributions are on top of the total cash-budget.

Project budget (k€)	Year 1	Year 2	Year 3	Year 4	Total
Personnel costs					
Salary PhD student	47	50	53	56,6	206,6
Research costs					
Consumables	6	6	6	4	22
Other	3	15	3		21
Total					249.6

Specification research budget

All necessary equipment and standard growth facilities for successful execution of the proposed program are already present in the in Plant Physiology Laboratory at the Wageningen University.

Standard consumables are required, in addition different reporter lines, mutant line, ecotypes and hormones need to be purchased (6k€/year). Moreover metabolomics experiments (LC-MS, GC-MS) need to be performed; 3 k€/year in year 1-3; additional costs will be covered from the budget of the host lab. And travel costs will be made (1.5K/year). RNA seq. experiments will be performed (with the associated data storage costs; 12 k€ in year 2). The budget excludes the in kind contribution of 18,75k€.

STATEMENTS BY THE APPLICANT

11. Statements by the applicant

NA I endorse and follow the Code Openness Animal Experiments (if applicable)

YES I endorse and follow the Code Biosecurity (if applicable)

YES I have completed this form truthfully

Name: Harro Bouwmeester
Place: Wageningen
Date: 16-6-2014

Please submit the application to NWO in electronic form (pdf format is required!) using the Iris system, which can be accessed via the NWO website (www.iris.nwo.nl). The application must be submitted from the account of the main applicant. For any technical questions regarding submission, please contact the IRIS helpdesk (iris@nwo.nl).

12. Curriculum vitae proposed PhD candidate

12a. Personal details

Title(s), initial(s), first name, surname: BSc, Joël Boerefijn
Nationality: Nederlandse

12b. Bachelor study

University/College of Higher Education: Utrecht University
Name Bachelor study: Biology
Specialisation: Plant Ecology / Molecular and Cellular plant biology
Non-compulsory courses followed: The Cell, Behavioural biology, Microbe-interactions, Paleo-ecology, Plants and their environment, Biodiversity and landscape, Environmental changes trough time, Plant adaptation and defence, Tropical ecology.
Thesis title: Tragedy of the commons in root competition
Thesis grade: 7,5
Date diploma: October 2012
Average grade: 6,93

12c. Master study

University: Utrecht University
Name Master study: Environmental Biology (track: Plant Biology)

Subjects year 1 (*List the subjects and courses followed*):

- Course, Plant and Their environment
- 12 months internship at the Utrecht University, at the Plant-Microbe-Interactions group led by Prof. C.M.J.Pieterse
    - Topic: Communication between *Arabidopsis thaliana* and the beneficial rhizobacterium *Pseudomonas fluorescence WCS417.*

Average grade year 1: 7,3

Specialisation (year 2; *Mention your chosen specialisation and list the subjects and courses followed*):

- Course, Molecular Plant Physiology and Biotechnology
- 8 months internship at the Glasgow University (United Kingdom), at the institute of Molecular, Cellular, and Systems Biology, led by Dr. A. Amtmann and Prof. M. Blatt
    - Topic: Root Architecture response of Arabidopsis thaliana to low nitrate, characterization of two nitrate transporters AKT-1 and NRT1.1 (CHL1-5).

Average grade year 2 (*If year 2 has not been completed yet, please provide the average grade so far*):
7.3

Graduation date: (*Date can be in the future*): September 2014
Title thesis: (*Proposed or completed thesis, please indicate status*): Trade-off between defence and growth in *Arabidopsis thaliana*.

- Status: Proposed

Thesis grade: NA

12d. Other academic activities

Please provide information about (extra)curricular academic activities the candidate has engaged in, for example, membership of committees or the involvement in the organisation of conferences (max 200 words).
I attended the annual Netherlands Organization for Scientific Research (NOW, ALW) annual Experimental Plant Science meeting in Lunteren in 2013 and 2014.

12e. Scholarships and prizes (if applicable)

Erasmus placement Scholarship for minor Research project in the United Kingdom.

12f. Scientific output of proposed candidate (if applicable)

If available, please mention below a maximum of 5 scientific publications or other relevant scientific output of the proposed candidate.

NA

12g. Candidate's motivation

Please provide your motivation for a PhD position (max 200 words)

After my bachelor biology there was an urge to obtain further knowledge in plant biology and I choose for the master program: Environmental Biology at the Utrecht University. But even now after almost two years of master program, two long internships, and a couple of courses I still have the idea that there is much more learn in plant biology. Not only on a theoretical level but also on a applied level, albeit I gained already a solid foundation in some molecular lab techniques during the two internships, I consider it necessary to acquire a better understanding of more techniques and new methods. Doing a PhD gives me this opportunity to on one hand gain more knowledge about a specific topic, and on the other hand to try and develop new (other) techniques. Getting acquainted with new techniques and methods is only one part of my motivation. The other part is that I want to contribute to the solution of the problem, how we can feed the growing world population in fifty years. With the research in my PhD I hope to obtain new information about molecular processes in plants that can help us to develop better crops in the near future.

12h. Title(s), initial(s), surname and university of the two references expressing support for the application on the two recommendation letters accompanying the application

Name: Dr. A. Amtmann
University: Glasgow University

Name: Prof. C.M.J.Pieterse
University: Utrecht University

13. Top 5 publications of the research group related to the proposed research

1. **Genetic variation in jasmonic acid- and spider mite-induced plant volatile emission of Cucumber accessions and attraction of the predator Phytoseiulus persimilis**
Kappers, I.F. , Verstappen, F.W.A. , Luckerhoff, L.L.P. , Bouwmeester, H.J. , Dicke, M. (2010)Journal of Chemical Ecology 36 (2010)5. - ISSN 0098-0331 - p. 500 - 512.
2. **Natural variation in herbivore-induced volatiles in Arabidopsis thaliana**
Snoeren, T.A.L. , Kappers, I.F. , Broekgaarden, C. , Mumm, R. , Dicke, M. , Bouwmeester, H.J. (2010)Journal of Experimental Botany 61 (2010)11. - ISSN 0022-0957 - p. 3041 - 3056.
3. **The biology of strigolactones**
Ruyter, C.P. , Al-Babili, S. , Krol, S. van der , Bouwmeester, H.J. (2013)Trends in Plant Science 18 (2013)2. - ISSN 1360-1385 - p. 72 - 83.
4. **Genetic engineering of plant volatile terpenoids: effects on a herbivore, a predator and a parasitoid**
Kos, M. , Houshyani Hassanzadeh, B. , Overeem, A.J. , Bouwmeester, H.J. , Weldegergis, B.T. , Loon, J.J.A. van , Dicke, M. , Vet, L.E.M. (2013)Pest Management Science 69 (2013)2. - ISSN 1526-498X - p. 302 - 311.
5. **Association mapping of plant resistance to insects**
Kloth, K.J. , Thoen, M.P.M. , Bouwmeester, H.J. , Jongsma, A.M. , Dicke, M. (2012)Trends in Plant Science 17(5).- p. 311-319.

14. Open Application letter (optional*)

Below a summary of the proposed project.