HORTINLEA
DIVERSIFYING FOOD SYSTEMS

"Diversifying Food Systems: Horticultural Innovations and Learning for Improved Nutrition and Livelihood in East Africa" (HORTINLEA)

JOINT INTERIM REPORT (SP2)

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FORMAL REPORT

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UNIVERSITÄTS KLINIKUM FREIBURG

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flame of knowledge and innovation

AVRDC
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Knowledge for better livelihoods

MRI Max Rubner-Institut

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icipe
African Insect Science for Food and Health

Description of activities/milestones
SP2: Development of integrated pest management strategies for the production of important vegetable crops in Kenya
PM1: Overview on severity and incidence of RKNs, phytoplasma and plant viruses A survey has been conducted and data are currently generated.
PM2: Diagnostic tools suitable for routine diagnosis of RKN, phytoplasma and viruses Laboratory analyses to identify viral pathogens in African nightshade were initiated and diagnosis for RKN has been started in laboratory experiments.
PM3: Recommendation of plant protection tools dependant on specific site conditions
PM4: Major sex pheromone components of M. vitrata identified and field tested Despite the delay in getting a subcontract with JKI rearing of Kenian *M. vitrata* populations have been established. Reference chemicals for chemical pheromone analysis have been ordered.
PM5: Effective botanical insecticides against *M. vitrata* identified Different strains of entomopathogenic funghi have been isolated and are currently tested against M. vitrata as well as Aphis craccivora.
PM6: IPM system for cowpea pests including *M. vitrata* developed and tested in the field IPM system will be developed in time
PM7: Development of IPM strategies for fungal diseases in selected indigenous vegetables Progress is on track. Experimental work on control of damping-off disease has started recently within the framework of a MSc thesis at JKUAT under supervision of Prof. Losenge
PM8: Monitoring and management of sucking pests and viral diseases in the agro ecosystem of indigenous leafy vegetables Progress is on track. Surveys in Kenya and Tanzania are finished. So far spider mites and aphids have been identified as major sucking pests on several leafy indigenous vegetables, i.e. leaf amaranth and African nightshade and Ethiopian kale. Both PhD students, J. Mworia (JKUAT) und D. Mureithi (icipe), started with experimental work. First results are available.

Root-knot Nematode pests: A survey study was carried out in order to determine Root-Knot Nematode disease severity and infestation in June 2014. It was conducted in selected farms (a total of 25) in Uasin Gishu, Bungoma and Kakamega counties which are the major African nightshade (AFNS) producing areas in Kenya. Root samples were collected in all farms for the analysis based on galling index and egg-mass index. Soil samples were also collected from each farm for determination of the physico-chemical parameters at the Kenya Agricultural Research Institute (ongoing). Preliminary findings indicate significant differences ($P \leq 0.05$) in disease severity between the counties with higher severities recorded in farms from Uasin Gishu and Bungoma counties relative to farms in Kakamega county (see Table 1 and Fig. 1 in Annex). Moreover, all the roots sampled were infected with RKN. Working activities of SP2 (PM1) also include the taxonomical assignment of nematodes obtained from AFNS-root systems (Western Kenya). A PhD student, Mr Shem Nchore (supervision Prof. Waceke, Kenyatta University), was visiting the institute for molecular and bioinformatical training at the division Phytomedicine (supervision Dr. Kube and Prof.

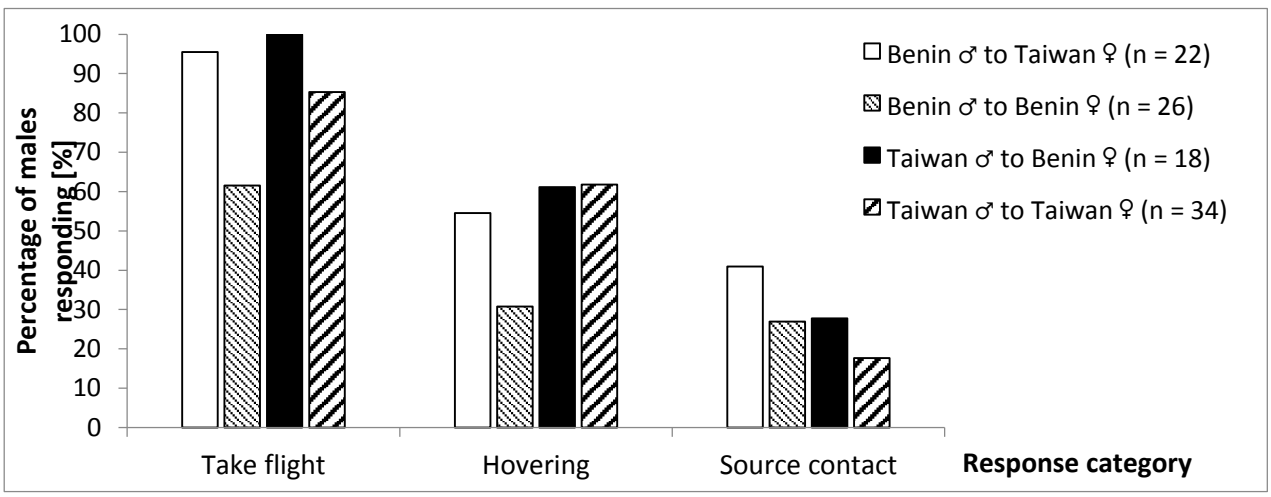
Büttner, Humboldt-Universität zu Berlin). In total, 100 nematodes were processed during the stay in the division Phytomedicine (03.11.- 11.12.2014). Results obtained during the stay were presented in a talk given by Mr. Nchore at the division. Furthermore, a first manuscript entitled: “Root-knot nematodes (Meloidogyne spp.) pests of African nightshades in Kenya” is in preparation. **(PM1-2)**. In order to determine severity/incidence of plant viruses and phytoplasma on AFNS in the field a survey was conducted in the same counties (with a total of 28 farms). 33 samples were taken to assess virus infection of cultivated indigenous/traditional African nightshade. To get an overview on incidence and severity of phytoplasma infection in African nightshade three individual plants were taken from 22 farms. Symptomatic plants were screened at Kenyatta University. Several packets of certified nightshade seeds were purchased from the Kenya Seed Company in Bungoma and will be analyzed in regard to seed borne viruses, and seeds were collected from different farmers who are not content with the yield. Laboratory analyses to identify viral pathogens in African nightshade were initiated. For that purpose mechanical transmission was conducted to different indicator plant species. Currently ELISA and PCR are applied to identify the pathogens. Results: Virus typical symptoms (mottling, dwarfing, deformed leaves, vein clearing, ringspots) started to appear on Nicotiana benthamiana and N. rustica five days after inoculation and became stronger from first to fourth plant passage within the biotest plants. Inoculated Nightshade plants showed only unspecific symptoms, similar to those of control plants. Virus infections of Nightshade crops were confirmed by a total of 70% positive ELISA (Table 2, see Annex) detections (predominantly CMV and/or potyvirus infections) in representative leave samples.

PM1-3: The possibility of phytoplasma infections in AFNS was examined by a molecular approach. Total genomic DNA was extracted from leaf material (3 leaves/ plant sample) applying the CTAB protocol. Samples were screened *via* PCR applying diagnostic universal phytoplasma primers (P1/P7) and subsequent nested-PCR (R2/F2). Reactions were examined for successful amplification via gel electrophoresis. Sequences derived from PCR products were determined via Sanger sequencing and analyzed. No phytoplasma infection was detected for the 50 examined AFNS plants. One sample produced an unspecific PCR product. In contrast, phytoplasma infection was verified for *Cynodon dactylon*, *Populus* sp. and *Catharanthus roseus*. Grasses, showing typical symptoms of the Bermuda grass white leaf disease, were infected by '*Candidatus* Phytoplasma cynodontis', as expected. Ornamental periwinkle plants are infected by '*Ca.* P. asteris', '*Ca.* P. solani' or '*Ca.* P. convolvuli' strains. One poplar-sample was obtained from a tree showing severe infection symptoms including light green coloring of leaves, impressive decrease in leaf size and proliferation. Diagnosis of a phytoplasma infection was verified but no assignment to a described '*Ca.* Phytoplasma' was possible so far. This survey shows the presence of several phytoplasma strains close to AFNS fields in Kenya detected so far only in other parts of the world. However, a possible impact of phytoplasma infections on symptom formation observed on African nightshade cultures could not be confirmed. The presence of phytoplasmosis in AFNS cannot be excluded due to the single sampling event. It is known that the pressure of this pest on cultures can vary between years. However, it remains unlikely that the observed symptoms indicate phytoplasma infections in AFNS in general. The development of improved tools for diagnosis of phytoplasmas (PM2, M2) and the evaluation of potential management tools (PM3, M3) is not necessary for this part in contrast to nematode pests and virus diseases. The phytoplasma part of SP2 fulfills the termination criterion TC-2b.

In order to assess farmers’ knowledge and awareness on problems caused by phytoplasma and viruses and to develop diagnostic tools a seminar with farmers cultivating African Nightshade was held in Sabatia, Kenya, leading to a fruitful discussion on factors affecting production. Farmers are neither familiar with virus induced characteristic symptoms nor diagnostic tools to analyze and

evaluate the health status of seeds. With the seminar farmers knowledge on plant diseases was expanded: Transmission modes of plant viruses were named and described exemplarily. The impact of plant virus transmission by seed, water and insect vectors on disease occurrence and yield losses was stressed. Certified seeds of Kenya Seed Company were provided to motivate farmers to adopt its use. A literature review on cultivation of African nightshade and occurrence of plant viruses and phytoplasma in this crop plant was carried out focusing on Eastern Africa. Diagnostic tools to identify these pathogens were evaluated in regard to its applicability for routine diagnosis and suitability for samples taken several days up to a fortnight prior analysis **(PM-3**).

PM-4: During the reporting period M. vitrata-pupae have been shipped from Taiwan, Thailand, Vietnam, Benin, and Kenya. Rearing has been established and rearing methods improved to limit viral infections. At the Julius-Kühn Institute analysis of single pheromone gland extractions of M. vitrata-females from Taiwan, Thailand, Vietnam, Benin, and Kenya have been conducted. The solvent for extraction has been hexane and the analysis were conducted using Gas chromatography coupled with mass spectrometry (selected ion monitoring). The following described pheromone compounds of M. vitrata have been identified: (E,E)-10,12-hexadecadienal, (E,E)-10,12-hexadecadienol, and (E)-10-hexadecenal, including their authentic stereoisomers: (E,Z)-, (Z,E)-, (Z,Z)-10,12-hexadecadienal, (E,Z)-, (Z,E)-, (Z,Z)-10,12-hexadecadienol, and (Z)-10-hexa-decenal. EE10,12-16:Ald and EE10,12-16:OH were found consistently in gland extractions in all M. vitrata – populations, their authentic isomers, E10-16:Ald and Z10-16:Ald, were not detected. The EE10,12-16:Ald represented the major part while EE10,12-16:OH represents the minor part. No significant differences observed in the blend ratio of EE10,12-16:OH/ EE10,12-16:Ald between all five populations. Wind tunnel assays have been conducted to test cross-attraction. In these experiments M. vitrata-populations from Benin and Taiwan where compared. Live female moths (positive control) and their gland extractions as source of pheromones where used. Male moth was given 10 min for taking flight. Failing this, the male moth was declared as a nonresponder. After taking flight, the male moth was given another 10 min until a positive response was observed or not. The male response was divided into the following behavioral categories: (1) taking flight but not orienting toward the source of stimuli, (2) oriented flight, ending in hovering in front of the pheromone source, and (3) pheromone source contact. Live females were caged singly inside a translucent, horizontally placed plastic cup, female moth was controlled constantly for showing the calling behavior -> extruding her ovipositor. One to three days old gland extractions were used as a source of stimuli. Five microliter of the hexane solution containing an equivalent of one female ovipositor washing were applied on a triangle of filter paper (no data for cross-attraction with gland extractions available yet). Males from



both populations did not show a higher positive response to their own females, there is cross-attraction when male can only chose one female.

Fig. Response of male *M. vitrata* towards female *M. vitrata* derived from populations of different origin.

During the period under review and following the optimization of bioassays as reported previously, 20 isolates were obtained from the icipe Microbial Bank and sub-cultured on Saboraud Dextrose Agar for bioassay against apterous adult *A. craccivora*. Initial screening has focused on using 9 isolates of *Metharizium anisopliae* and *Beauveria bassiana* (ICIPE 07, 10, 18, 20, 30, 62, 63, 69 and 78). The isolates were tested at a standard concentration of 1 x 10^8 conidia ml^{-1} by spraying 10 ml of the conidial suspension on the aphids on leaf disc using the Burgerjon Spray tower. Results showed that all these isolates induced significant levels of mortality to *A. craccivora* (ranging from 46 to 88%). However, ICIPE 62 and 69 are by far the best performing isolates, with 88 and 72% of mortalities respectively. Lethal time to 50% mortality ranged between 2.5 and 4 days, with the shortest time obtained for ICIPE 62. For all 9 Isolates, upon death, diagnosis revealed profuse growth of mycelia and spores of *M. anisopliae* from the surface of cadavers. Inoculum levels arising from individual dead aphid ranged from 1 x 10^4 to 1 x 10^6 conidia ml^{-1}, with ICIPE 62 producing the highest quantity of inoculum **(PM4-6**). Nimbecidine®, a commercial oil-based neem pesticide containing azadirachtin (300 ppm) as the active ingredient was tested against apterous adult aphids in the laboratory. One hundred apterous adults were placed in glass Petri dishes (10 cm diameter). Insects were sprayed from an acrylic spray chamber fitted with a hand sprayer located 50 cm from the glass petri dishes. A water sensitive paper was used to assess the distribution of the spray droplets from the nozzle of the hand sprayer prior to conducting bioassay. Total sprayer deposit was 2213 ± 56 drops cm^{-2} and the pattern of coverage indicated that droplets were evenly dispersed across the spray arena and thus expected to impinge on aphids on the Petri disc located in the spray arena. Five milliliter of the neem solution (300, 900, 1500, 2100, 2700, and 3100 ppm azadirachtin) or sterile distilled water was applied to aphids in the spray arena in groups of 20 with five replications per azadiractin concentration. Mortality of aphids was determined after 24 h by examining each dish and noting aphid activity. Insects not responding to a probe were noted as dead. Results showed that mortality in the control was 5.2%. Response to the botanical pesticide was concentration dependent. The estimated lethal concentration values in ppm were: LC_{10} (411.7 – 95% fiducial limit: 473.8-484.8); LC_{50} (2854.2 – 95% fiducial limit: 2093.2-2952.6) and LC_{90} (2878.9 – 95% fiducial limit: 2714.2-2987.5). Ongoing screening is looking at the potential of pyrethrum botanicals commonly used by growers for aphid control. Once a potent, botanical has been identified, they will integrated with candidate biopesticides in a field suppression to be undertaken in September 2015 **(PM5-6**).

PM-7: Development of IPM strategies for fungal diseases in selected indigenous vegetables: As important fungal disease in the nursery of African nightshade (AN) and Ethiopian kale (EK) damping-off disease caused by Pythium aphanidermatum (Edson) Fitzp. 1923 (Oomycetes, Peronosporales), was identified. A MSc student (supervised by Prof. Losenge JKUAT) started to investigate the efficiency of antagonistic soil fungi (Bacillus subtilis and Trichoderma asperillum) for control of damping-off disease in both vegetables. First results show, (1) that both species of antagonistic soil fungi were able to increase survival of AN and AK substantially and increase plant growth, (2) that effects in nursery of EK were stronger than for AN, and (3) that plant growth depends in both cases on specific dose-response relationships. - Ongoing experiments are necessary to add support and verify current promising results in practice.

PM-8: Field surveys in important vegetable growing areas in Kenya and Tanzania were done during two field seasons by involved PhD students (Jackline Mworia, JKUAT; Daniel Mureithi, icipe) African

nightshade and leaf amaranth were identified as widespread indigenous vegetables. Other popular vegetables are Ethiopian kale, spider plant and jute mallow. During field surveys current growing practice and plant protection strategies have been identified. Both show high variation and plant protection in general is based on pesticide use. Relevance of pest arthropods in different seasons and growing regions varied slightly. Some species were only of regional importance. So far aphids (Aphis gossypii, Myzus persicae, Aphis citricola), fleabeetles (Epitrix silvicola, Phyllotreta spp.) and red spider mites (Tetranychus urticae, T. evansi) are identified as most important pest species on African nightshade, while mites, aphids, beetles were most abundant species groups on leaf amaranth. In contrast Ethiopian kale was most frequently attacked by lepidopteran caterpillars, aphids and leaf beetles. Pest and natural enemy species are currently identified to species level by the Kenia Museum. Leaf samples with viral symptoms were collected and virus identities are currently investigated at LUH. So far PVMV (Pepper Veinal Mottle Virus), CVMV (Chilli Veinal Mottle Virus) and a third so far unknown potyvirus species were detected in African nightshades. Antiserum for ELISA tests with both known potyvirus are available at DSMZ. The third species still has to be identified. PVMV as well as CVMV are known to be aphid transmitted. Further investigations concentrate on infection path ways for pests, natural enemies and virus on the farm level (Daniel Mureithi, PhD at icipe) and the impact of vegetable growing practice on pests (Jackline Mworia, PhD at JKUAT) First lab and greenhouse experiments on host preference of spider mites (Tetranychus evansi) for African nightshade at specific fertilizer regimes were developed by Ms. Mworia at JKUAT. Preliminary results show that spider mites prefer in the long run African nightshades with optimal fertiliser supply, respectively best conditions for reproduction. In contrast spider mites prefer leaf discs from plants with moderate fertilizer regime and also showed highest reproduction rates. With ongoing experiments, it will be tried to characterize the mechanisms in more detail. Design of specific field experiments for infection pathways on farm level has been finished.

The samples collected during the previous reporting period (corresponding to the long rainy season) were processed and sent for identification in this reporting period. While identification to species level is still ongoing, results indicate the importance of various pests in nightshade and amaranth production in the 15 counties surveyed, however their importance varied based on crop and county. For instance on amaranth, while Caterpillars, bugs, aphids and stem weevils were recorded as important pest countrywide, spider mites were important only in the coastal province (Mombasa, Kilifi and Lamu counties) and Nyanza province (Nyamira, Kisii and Kisumu counties), and whiteflies were important only in Kirinyaga county in Central province. The key Lepidopteran pests include *Spoladea recurvalis*, *Spodoptera exiga, S. Litoralis* and *Udea ferrugalis*. Aphids species are mainly composed of *Myzus persicae* representing 74% of aphids sampled processed so far, followed by *Aphis gossiipy* (25%). So far the only species of stem weevil identified is *Hypolixus nubilosus*. Species identification is ongoing for bugs. On nightshade, the key pests countrywide are aphids, flea beetles and mites. Unlike in the case of amranth, *Aphis gossiipy* consitutes by far the most abundant aphid species on nightshade, representing 81% of aphid samples processed so far, followed by *Myzus percicae* (16%). Flea beetle species include mainly *Epitrix silvicola*, *Phyllotreta* spp. and *Luperodes quaternus*. A second round of survey targetting the short rain season was carried out from October to November 2014. A total of 13 counties were surveyed (Nyamira, Kisumu, Kilifi, Mombasa, Kajiado, Kiambu, Kirinyaga, Kakamega, Busia, Embu, Machakos, Tranzoia, Narok). The samples are still being processed and material submitted to the museum of Kenya for identification. In general, while the pest composition remained the same as in the 1st season, except in Nyamira county where pest pressure was higher in the second season compared to the first season, in all other counties, the

pest pressure was far lower in the second season compared to the 1st survey carried out in the long rainy season from February to May 2014 **(PM-8)**.