Fytosanitaire actie in het land van oorsprong

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Inhoudsopgave

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1 Introductie

1.1 Aanleiding

Internationaal worden fytosanitaire regels steeds strenger en wordt het door moderne technieken mogelijk bij een steeds lagere detectiegrens plantenziekten waar te nemen. Bedrijfsleven en overheid hebben er belang bij de versleping van ziekten en plagen te voorkomen door maatregelen zoveel mogelijk aan de bron te nemen, of anders op de meest effectieve en efficiënte plaats in de keten. Hierdoor kunnen grensinspecties op termijn beperkt worden en wordt het geheel betrouwbaarder. Toenemende inspectielasten en logistieke verstoringen worden hiermee voorkomen. Nieuwe technologie kan de bestaande visuele partij-inspecties van de overheid versterken (in combinatie met kostenbeperking) en ook het bedrijfsleven helpen zelf kostenefficiënte maatregelen te nemen en risico's te verminderen.
Een bijkomend voordeel kan zijn dat een intensievere inzet van technologie (na de oogst) om te voldoen aan fytosanitaire eisen, bijdraagt aan het verminderde gebruik van gewasbeschermingsmiddelen.

1.2 Probleemstelling

De samenwerking richt zich op het verder ontwikkelen en toepassen van nieuwe en bestaande technologieën in het land van herkomst waardoor producten fytosanitair veiliger worden. Dit moet leiden tot minder afkeuringen tijdens import van producten in Nederland en minder (intensieve) inspecties in Nederland (EU) mogelijk kunnen worden. Door samenwerking in de gouden driehoek van overheid, bedrijfsleven en onderzoek van zowel Nederland als in het land van herkomst is het doel tot ontwikkeling en acceptatie van nieuwe non- destructieve technieken te komen waardoor de toepassing van deze technieken door het bedrijfsleven in land van herkomst draagvlak bij alle partijen heeft.
Er is sprake van een sterke interactie met het project CATT: duurzame aanpak van plagen in de handel".
De CATT methodiek is één van de nieuwe kansrijke technologieën om betere fytosanitaire garanties te krijgen. In dit project wordt vooral aandacht besteed aan fytosanitair veilige exportstromen.

1.3 Doelstelling(en) van het project

Dit project richt zich op het bevorderen van de eigen verantwoordelijk van het bedrijfsleven bij het ontwikkelen van alternatieve risico beperkende maatregelen. Het doel van het project is het bevorderen van samenwerking tussen exporterende landen en Nederland. Uiteindelijk meer verantwoordelijkheid bieden/vragen aan bedrijfsleven (in land van oorsprong) om m.b.t. non-destructieve methoden ervoor te zorgen dat tijdens officiële inspecties (zowel in land van herkomst als in NL) minder Q-organismen worden aangetroffen en minder afkeuring noodzakelijk is. Ook samenwerking tussen betrokken partijen is nadrukkelijk doel (conform de oorspronkelijke BOCI-projecten).
De samenwerking richt zich op het ontwikkelen van toepassingen van (nieuwe maar bestaande) technologieën in het land van herkomst waardoor producten fytosanitair veiliger worden. Dit moet leiden tot minder afkeuringen tijdens import van producten in Nederland waardoor minder afkeuringen noodzakelijk zijn en minder (intensieve) inspecties in Nederland (EU) mogelijk kunnen worden. Door samenwerking tussen de gouden driehoeken (overheid, bedrijfsleven en onderzoek) van zowel Nederland als land van herkomst is het de bedoeling dat tot ontwikkeling en acceptatie van nieuwe non destructieve technieken wordt gekomen waardoor de toepassing van deze technieken door het bedrijfsleven in land van herkomst draagvlak bij alle partijen heeft.

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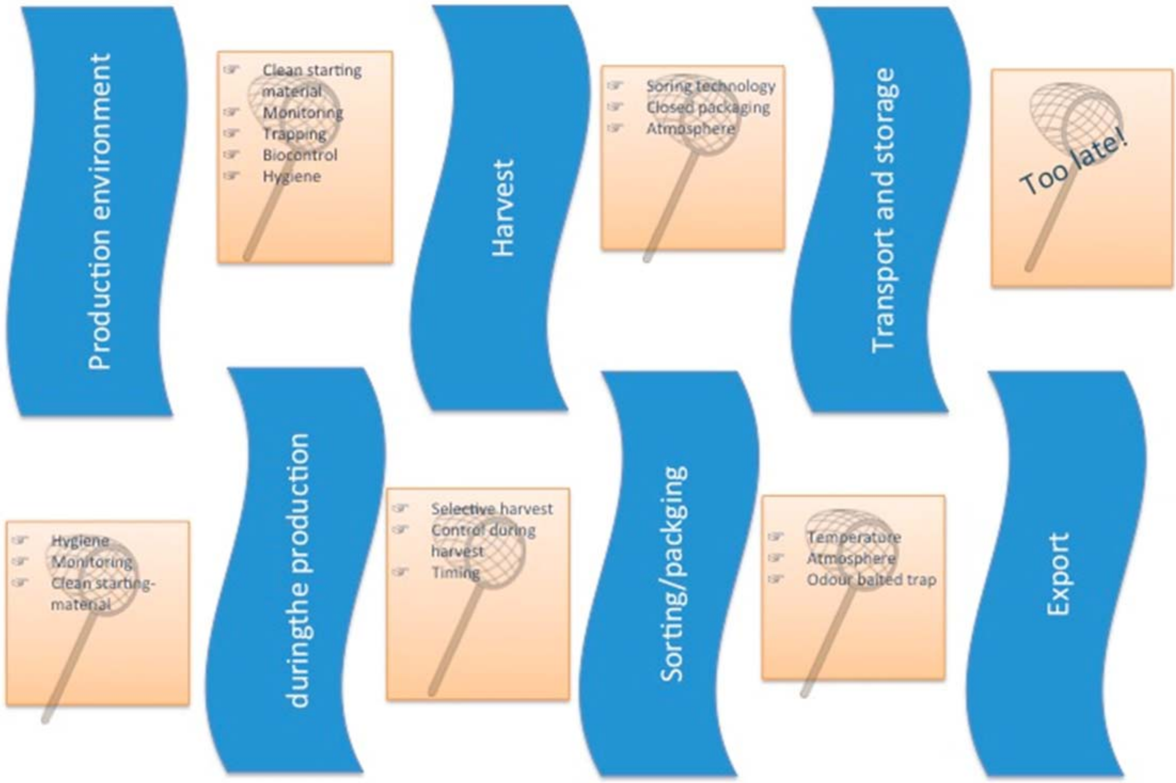

Figuur 1 Fytosanitaire maatregelen in de productie keten.

1.4 Doelgroep(en)

Bij de keuze voor pilotlanden is rekening gehouden met de aard van de problemen, de bilaterale verhouding, de verwachte kracht van onderzoeksinstituten en het bedrijfsleven en de overheid, de intensiteit van de contacten tussen bedrijven in NL en het pilotland, en de verwachte haalbaarheid van de inzet van technologie i.r.t. organismen en gewassen.
Voor 2013 betreft het de volgende quarantaine problemen:

1. *Guignardia* (Citrus Black Spot) in citrusfruit vanuit Brazilië en Argentinië.
   Guignardia is internationaal een ernstig handels verstorend element. De verwachting is dat een lage besmetting van citrus met nieuwe technologie gedetecteerd kan worden en kan gebruikt kan worden bij de beoordeling van de exportwaardigheid of als onderdeel van het sorteerproces.
2. Boorvliegen (*Tephritidae*) in mango's vanuit Brazilië.
   De verwachting is dat beginnende aantasting van boorvliegen met behulp van X-ray eenvoudig te detecteren is en dat dit kan dienen als informatie voor de beoordeling van de exportwaardigheid of als onderdeel van het sorteerproces. Er is mogelijk op termijn van enkele jaren voordeel te behalen in het aantal noodzakelijke controles.
3. *Anoplophora* spp. (Aziatische boktorren) in Bonsaiboompjes uit China.
   De inspectiekosten om de boktor te detecteren zijn erg hoog (doorsnijden van materiaal) en de boktor is een groot fytosanitair risico. De verwachting is dat met X-ray de holte in de boompjes snel gevonden kan worden en op die manier zelfs tijdens sorteerwerkzaamheden apart genomen kunnen worden. Daarnaast is de CATT methode een mogelijk alternatief voor methylbromide of hete stoom behandeling (ISPM15). Indien deze technieken succesvol blijken dan helpt dat de bilaterale betrekkingen tussen NL en China te versterken.
4. *Thrips palmi* in orchidee (Dendrobium) uit Thailand
   Uit NVWA rapportages blijkt dat er al jaren een vrijwel constant percentage is van afkeuringen op

Thrips palmi. Dit wijst op een constante infectiedruk met als risico dat er een uitbraak komt. Daarnaast is Thailand een belangrijke producent en exporteur maar is de fytosanitaire organisatie slecht geregeld en zijn producten uit Thailand steeds een punt van aandacht. Samenwerken bij het ontwikkelen van fytosanitaire systemen is op zichzelf al van belang. Zowel tripsbeperkende maatregelen tijdens de teelt als alternatieven voor methylbromide voor sanitaire en efficiënte inspectie aan het eind van de keten verdienen aandacht.

Omdat er samengewerkt moet worden in de gouden driehoek overheid, onderzoek, bedrijfsleven van Nederland en van derde landen is coördinatie van groot belang. Betrokken partijen zijn, naast de onderzoekers, de bedrijven in de sectoren en de internationale vertegenwoordiging van EZ. De 'overall' coördinatie van het project wordt gedaan door een groep van bedrijfsleven en overheid: Inge Ribbens (Frugi Venta), Paul van der Zweep (VGB), Henk Schollaart (EZ-PAV), Annet Zweep (EZ-DAK). Aan deze groep worden de voortgang, go/no go momenten voorgelegd en planning/resultaten van internationale bezoeken besproken. Deze groep legt ook de verbinding met het CATT-project.

2 Werkplan

2.1 Aanpak en tijdspad

1.1.1 2.1.1 De werkplannen voor Guignardia op citrusvruchten

- Een meer uitgebreid onderzoek zou plaats vinden op de productketen van citrusvruchten en export, zowel in Argentinië en in Brazilië. We zullen infromatie verzamelen over de besmette percelen, de rol van klimaat, ?, de teelt, verpakking, de inspectie, opsporing en het vervoer van de citrusvruchten in Argentinië en Brazilië, om te weten te komen wat de verschillen in infectie beinvloedt.

- Het 2de deel van het werkplan is het uitvoeren van een pilot -experiment in Nederland. Voor deze experiment citrusvruchten zonder symptoom uit een veld in Brazilië met een hoge CBS infectie zal worden overgedragen aan Nederland. We zullen inductie test uit voeren met het doel de ontwikkeling van het symptoom te versnellen. Tijdens het hele proces van de citrusvruchten zal worden gemonitord met speciale camera's. Daarna zullen de symptomen geanalyseerd worden met moleculaire technieken. Met deze methode hopen we kunnen de symptomen in een vroeg stadium te kunen detecteren , wanneer het nog niet mogelijk is deze visueel te kunnen waarnemen .
- In de 3e fase zal een bezoek zowel naar Brazilië en Argentinië worden gedaan om het onderzoek te bespreken met de lokale partners en verder aan te vullen. Ook de resultaten (indien aanwezig) van de pilot experimenten zullen bediscussieerd worden.

2.1.2 Werkplan voor fruitvlieg in mango uit Brazilië

Zouden maatregelen genomen voor de export naar de VS toegepast kunnen worden voor de export naar Europa? Warm water behandeling van het product na de oogst is zeer effectief, maar beïnvloedt logistiek, kosten en fruitsmaak . Een monitoringsprogramma is veel goedkoper en lijkt makkelijker te implementeren. Daarom stellen wij voor om de mogelijkheden van de invoering van een gecertificeerd systeem van toezicht te verkennen.
• Stap 1 , eerste maanden van 2013. Verkennen haalbaarheid, uitwerking van de contouren van een monitoringprogramma. Vragen die worden beantwoord in deze fase zijn： 1）Wat zijn de vereisten van een monitoringprogramma? 2）Wat is het verwachte effect op de frequentie van fruitvliegen in geëxporteerde mango? 3）Hoe kan het technisch worden georganiseerd? 4）Hoe kan het worden gecertificeerd? Wat zijn de commerciële implicaties? 5）Wat is / moet de rol van de overheid, importeurs, exporteurs, andere partijen zijn? Wat zijn de kosten?

• Stap 2. Bespreek het overzicht met NL stakeholders. Nemen beslissing over go / no go voor stap 3.
• Stap 3. Organiseer een bijeenkomst om het monitoringsysteem uitwerken met Braziliaanse en Nederlandse stakeholders . Zo'n vergadering vindt idealiter plaats tussen augustus en oktober , wanneer de meeste van de export naar de VS plaatsvindt en het systeem kan worden gezien
Als u bij stap 2 een monitoring systeem niet haalbaar lijkt, zullen alternatieve leads worden onderzocht .

2.1.3 Werkplan voor boktorren in bonsai planten uit China

Fase 1. Een stage student begeleiden voor de ontwikkeling van val systeem voor volwassen *Anoplophora chinensis.* De stage zal plaastevinden bij het onderzoek in Beijing Forestry University.
A. Ontwerp van experimenten met vallen met lok stof voor *A. chinensis*

- Selecteren verbindingen uit de literatuur die aantrekkingseffect zou hebben.
- Testen van allerlei combinaties van de stoffen.
- Toetsen aantrekkingseffect in het lab en in de veld.

Fase 2. Pilot onderzoek dodingseffect van CATT op insecten (zo als boktorren) binnen hout galerij. Lokale boktor soorten zullen verzameld en getest worden onder diverse CATT condities om de dodingsvermogen te testen, deze simuleert de situatie voor andere boktor soorten zo als *Anoplophora chinensis* in hout.

Fase 3. Samen met Philips China om de mogelijkheid van Röntgen of CT scanning apparaten de galerij en larven van boktorren te detecteren.

2.1.4 Werkplan voor *Trips palmi* problematiek in Thailand.

Fase 1. Organiseren van een symposium met stakeholders in Thailand, waarbij telers, koepelorganisaties, onderzoekers en inspectie mensen de fytosanitaire situatie bespreken en mogelijkheden ter verbetering verder verkennen. Nadruk zal gelegd worden op implementatie van IPM en nieuwe technologieën om milieuvriendelijker maar wel effectief kwalitatief goede en thripsvrije export van orchideeën te garanderen. Monitoring systemen, vervanging van Methylbromide door CATT, en snelle moleculaire detectie bij inspectie staan daarbij op de agenda.
Ter voorbereiding van dit symposium worden de technische mogelijkheden in Nederland zo goed mogelijk verkend.

Fase 2. Het symposium zelf waarbij 2 onderzoekers Nederland in samenwerking met de Thaise ambassade het symposium zullen leiden, presentaties houden, mogelijkheden nagaan, bedrijven zullen bezoeken en afspraken maken over vervolgacties.

3 Resultaten en doorwerking

3.1 Citrus Black Spot (CBS) Guignardia citricarpa

- Er is een vragenlijst opgesteld ten behoeve van een grondige inventarisatie van de gehele keten van citrus-productie en –export. Deze is naar Brazilië en Argentinië verzonden. Respons wordt verwerkt.
- Dit onderdeel is voorbereid met een Braziliaanse citrus onderzoeker maar kan niet worden uitgevoerd i.v.m. gebrek aan medewerking van de Braziliaanse autoriteiten. Zij lieten de landbouwattache weten niet mee te willen werken aan dit project. Wellicht speelde onvoldoende communicatie richting de autoriteiten hierin een rol. Daar het seizoen inmiddels is verstreken kan geen materiaal meer voor dit pilot experiment worden verzameld.

- Uit de intercepties van Guignardia door NVWA, werden 100 citrus vruchten met pre-symptomen gevolgd voor verdere symptoom ontwikkeling visueel beoordeeld door een NVWA expert en vergeleken met een moleculaire LAMP techniek (Bijlage 1). De LAMP techniek lijkt perspectiefvol voor de detectie van Guignardia tijdens uitgangsmateriaal productie, tijdens citrus productie en in de naoogst fase.

- Er is in mei 2013 een reis uitgevoerd naar Argentinië. Tijdens die reis kwamen intercepties vanuit Argentinië naar voren. Het aantal intercepties is inmiddels boven de door de EU gesteld grens van 5 intercepties. Argentinië heeft inmiddels een missie naar Nederland uitgevoerd (EZ en NVWA). Argentijnse stakeholders staan open voor samenwerking op het gebied van onderzoek en pilots.
- Er werd een Teleconferentie gehouden met de landbouwattachés uit Brazilië en Argentinië om het project beter te communiceren. De hoop is om de pilot experiment materiaal vanuit Argentinië geregeld worden.

3.2 Boorvliegen in Mango

Aantal interviews en gesprekken werden gevoed om de volgende situaties helder te maken (bijlage 2):

- Aanscherpen beeld importproblematiek boorvliegen in mango, zowel wat Q-soorten betreft als wat niet-Q soorten betreft.
  - Herkomsten
  - Vergelijkbare problematiek met andere producten? Tafeldruiven uit Brazilië en Chili bijvoorbeeld?
- Beeld van werkwijze importinspecties (en exportinspecties in landen van herkomst)

- Bespreken wat kansrijke oplossingsrichtingen zijn: certificeringssystemen in land van herkomst? Wat kunnen we leren van jullie ervaring? Andere oplossingsrichtingen.
- 2013: meer boorvliegen aangetroffen dan in 2012. Rayon Zuid West: 30 vastleggingen boorvlieg wat inderdaad Q-boorvlieg bleek tot 15 november Ook Bactrocera en Anasthrepha kwamen voor. Statistieken voor aantreffingen en herkomsten worden bijgehouden voor jaarlijkse rapportage.
- 2012: vaak boorvliegen aangetroffen in mango bij Cool Control (veelal C. Capitata, MedFly). In 2013 bij Mooy Logistics, Roveg, ADB (allen service verleners, dus niet dé belanghebbenden.
- Herkomsten: meer aantreffingen bij ready-to-eat concept mango's. Deze komen vooral uit West Afrika, per vliegtuig. Typisch voor deze mango's uit West Afrika: kleine telers, verschillende telers per pallet, soms slechts enkele dozen per teler. Deze zijn rijper geoogst en daarom meer kans op infectie door boorvlieg in land van herkomst en ontwikkeling symptomen door boorvlieglarve in het logistieke proces.
- De bulk aan mango's uit bijv. Brazilië komt van grote telers. Dit kunnen meerdere containers van dezelfde telers zijn voor één zending naar één importeur.
- Andere gewassen met boorvliegen: ook Guave, Carambola (sterfruit), Pomelo (China, tevens 'Ten Spot', was eerder reden voor vasthouden partijen om te checken op 'Black Spot'. Nu niet meer, maar NVWA wil wel monsters ontvangen om te monitoren of Black Spot voorkomt. Hete pepers: was niet inspectiewaardig voor Q-organismen. Bij controle bleek 8% besmet met boorvliegen. Wordt daarom nu wel een inspectiewaardig product.
- Geen aantreffingen op tafeldruiven.
- Meerwaarde certificering mango's irt boorvliegen:
- niet veel kansen in certificering als stimulerings/of borgingsinstrument
- Niet direct aanleiding inspecties te wijzigen
- Pas als langere tijd minder aantreffingen voorkomen, kan intensiteit inspecties verlaagd worden. Dus na-ijl-effect als certificering minder besmettingen/infecties tot gevolg heeft.
- Wel direct kostenbesparing bedrijven bij minder aantreffingen.

3.3 Anoplophora boktor in houtig plant materiaal



- Een student-stage wordt begeleid om lokstof van *Anoplophora chinensis* te ontwikkelen voor de monitoring van volwassen boktorren. Lab en veld experimenten worden uitgevoerd in Beijing Forestry University en Pingtan, Fujian. Een van de getoetste mengsels van plant geuren toont aantrekkingseffect voor de boktorren in het lab, hoewel dit effect niet werd aangetoond in de veld. De studie is beschreven in een stage verslag van de student (bijlage 3). Goede samenwerkingsband werd opgebouwd tussen WUR, NVWA, Beijing Forestry University en Fujian Agriculture and Forestry University. De resultaten werden gepresenteerd op de 2nd International Congress on Biological Invasions, Qingdao, China (bijlage 4).
- In 2013 is een Letter of Intent ondertekend door de Wageningen UR (Plant Science Group) en de CAIQ (Chinese Academy of Inspection and Quarantine) (bijlage 5). Het gaat om een intentie voor onderzoekssamenwerking tussen WUR en CAIQ op gebied van toepassing van detectie methoden

(Röntgen en/of CT) en CATT techniek in quarantaine plaag bestrijding van Aziatische boktorren, onder andere in bomen en in verpakkingshout.

- Een businessplan voor samenwerking met het Institute of Inspection Technology and Equipment (onder ACIQ) voor onderzoek en toepassing van de imaging detectie techniek en CATT techniek zal worden opgesteld. Het IITE wordt bezocht door onderzoekers uit Wageningen UR en een discussie wordtgevoerd over de vorm, inhoud en financieringsmogelijkheden van samenwerking. International Plant Protection Convention (IPPC) wil graag dat toepassing van chemische bestrijdingsmethoden in de komende jaren verminderd wordt, waardoor een fysische methode zoals CATT duidelijk prioriteit heeft. IITE wilt dit jaar al starten met de onderzoek, als voorbereiding voor de aanvraag voor een internationale samenwerkingen project in 2014 bij de International samenwerkingsprogramma bij het Chinese Ministerie van Wetenschap en Technologie. Ook een bezoek aan het Controlled Atmosphere bedrijf Fruitong is uitgevoerd door Wageningen UR onderzoekers, om de mogelijkheden om CATT als technologie in China te kunnen introduceren te bespreken (bijlage 6).
- Thuja boomstammen geïnfesteerd met de Japanse thujabastkever (*Phloeosinus rudis*) zijn behandeld met 10 CATT condities; bij 35 en 40 ˚C zijn diverse CO_2/O_2 combi's gedurende 24 of 48h behandeld (Bijlage 5). Er zijn geen effecten van CATT aan de larven, poppen en adulten aan deze kever aangetoond. Blijkbaar heeft de doding van deze thujabastkever strengere CATT behandeling nodig, b.v. hoger temperatuur, langer behandelduur en mogelijk een hoger CO_2 en lagere O_2 concentraties (bijlage 7).

- Een Wageningen UR onderzoeker en NVWA medewerker zullen in oktober IITE bezoeken om over details van de uitvoering van de samenwerking te bespreken.


3.4 Thrips palmi op orchideeën in Thailand



- In november 2013 is er een workshop gehouden samen met de orchideeën telersorganisatie en de inspectiedienst om eerdere opties voor verbetering van een (bij export) tripsvrije orchideeën keten uit te werken (programma van de bezoek zie bijlage 8). Uit het vorig verkennend bezoek is duidelijk geworden dat (1) de huidige beheersingstrategie in de teelt goed werkt om *Trips palmi* grotendeels te onderdrukken maar het is sterk chemisch met een ongewenste input aan bestrijdingsmiddelen en (2) dat voor export overal op export bedrijven begassingsinstalliaties voor methylbromide aanwezig zijn die effectief zijn om orchideeën bij export zo goed als trips vrij te krijgen. Desondanks wordt er nog een veel tijd en moeite geinvesteerd om ook op de luchthaven nog inspecties te doen.

- Gewenste verbeterpunten voor een minder chemisch georiënteerde keten met gelijke effectiviteit:
  - verkenning met telers over IPM maatregelen in de teelt, met name monitoring en mogelijk ook biologische bestrijding. Bezoeken werden gebracht aan zowel traditionele sterk gediversifieerde telers als aan hypermoderne gespecialiseerde telers waaruit naar voren komt hoe gevarieerde de teeltomstandigheden zijn, hoe intensief de chemische bestrijding is om trips op laag niveau te houden en hoe lastig het zal zijn om milieuvriendelijker methoden te implementeren. Toch lijken er openingen en is er interesse voor verbeterde monitoring met vallen en geurstoffen en teopassing van biologsiche bestrijding (bijvoorbeeld met lure en kill).
  - vervanging van methylbromide door een andere effectieve methode (bv CATT) die de kwaliteit van de bloemen garandeert of zo mogelijk verbeterd (methylbromide verlaagd de kwaliteit) heft zeker aandacht. De eerste CATT experimenten in Wageningen (in samenwerking met een importeur) lieten zien dat orchideen die redelijk tot goed doorstaan en de trips gereduceerd wordt. De in Thailand gepresenteerde resultaten werden goed ontvangen waardoor initiatieven worden genomen om dit ook in Thailand verder uit te werken (bijlage 9).
- bemonstering- en detectiemethodes (moleculair) die de nacontroles bij de inspectie minder intensief maken. Moleculaire detectie van trips bij de inspectie (op de luchthavens) lijkt vooralsnog logistiek niet inpasbaar vanwege de hoge verwerkingssnelheid.




4 Coördinatieoverleg

Omdat er samengewerkt moet worden in de gouden driehoek overheid, onderzoek, bedrijfsleven van Nederland en van derde landen is coördinatie van groot belang. Betrokken partijen zijn naast de onderzoeker de bedrijven in de sectoren en de internationale vertegenwoordiging van EZ. De 'overall' coördinatie van het project vervult een groep van bedrijfsleven en overheid: Inge Ribbens (Frugi Venta), Paul van der Zweep (VGB), Henk Schollaart (EZ-PAV), Annet Zweep (EZ-DAK), Jan Schans (NVWA). Aan deze groep worden de voortgang, go/no go momenten voorgelegd en planning/resultaten van internationale bezoeken voorgelegd. Deze groep legt ook de verbinding met het CATT-project.

Mevrouw Homa Ashtari van de EZ heeft hulp verleend bij de communicatie met de landbouwattache in Brazilië en Argentinië. Onderzoekers van WUR en EZ beleidscommissie heeft een telefonisch overleg met de landbouwattachés uit deze twee landen over het project toegelicht. Gezien de negatieve reactie van de Braziliaanse autoriteit, de advies van de beleidscommissie is om de Guignardia onderdeel in Argentinië uit te voeren. Dit speelt ook voor het fruitvlieg onderdeel, een mogelijk alternatief is Thailand, waar het contact met alle partijen goed loopt en waar, naast *Thrips palmi*, mango fruitvlieg een actueel probleem is.

5 Monitoring en evaluatie

Het project voorstel en plan werd diverse keren besproken in een fytosanitair begeleidingscommissie waarbij overheid, inspectie, bedrijfsleven betrokken zijn. Ook is een bestuursgroep gevormd om een aantal keer per jaar coordinatieoverleg te houden om de voortgang van het project bespreken.

6 Publicaties

Link	***Titel***	***Jaar***	***Auteur(s)***
http://www.icbi2013.org/web/NewsDetail.aspx?tlD=41	Development of Odour-Baited Trapping System for the Citrus Longhorn Beetle *Anoplophora chinensis*	2013	Qiu, YT, Verbon, E, Lu, PF, Zhang, FP; Guang, Liang H, Luo YQ & Loomans, A

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9 Externe teamleden alleen Contactpersonen EZ

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10 Samenvatting

Dit project richt zich op het bevorderen van de eigen verantwoordelijk van het bedrijfsleven bij het ontwikkelen van alternatieve risicobeperkende maatregelen. Het doel van het project is het bevorderen van samenwerking tussen exporterende landen en Nederland. Uiteindelijk meer verantwoordelijkheid bieden/vragen aan bedrijfsleven (in land van oorsprong) om mbt non-destructieve methoden ervoor te zorgen dat tijdens officiële inspecties (zowel in land van herkomst als in NL) minder Q-organismen worden aangetroffen en minder afkeuring noodzakelijk is. Ook samenwerking tussen betrokken partijen is nadrukkelijk doel.

De voortgaan voor de volgende vier cases zijn gemaakt:
Citrus black spot (CBS)
- Questionnaire Argentina / Brazilië
- Reis Argentina (Mei 2013) bezoek congres Argentinean Citrus Congress
- Pilot experimenten uitgevoerd voor het analysen met monsters uit intercepties van NVWA met LAMP en phenotyping
- Teleconference landbouw attaches Argentina / Brazilië
- Via landbouw attache om ondersteun te zoeken bij Argentijnse overheid
- Missie naar Argentina / Brazilië uitgesteld

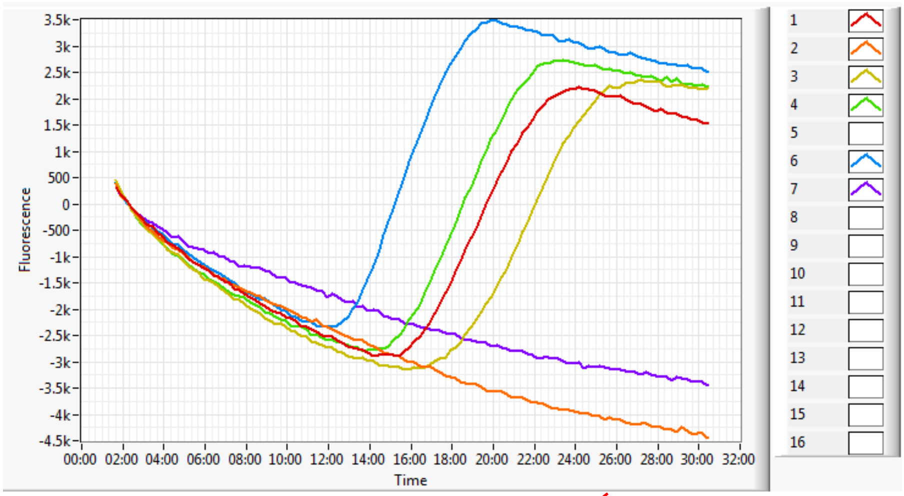
China citrus long horn beetle (CLB)
- Supervisie van een studente stage. Lab en veld proeven uitgevoerd om lokstoffen voor de volwassen CLB.
- Een Letter of Intend ondertekend tussen WUR en CAIQ voor samenwerkingsonderzoek op de detective en behandeling van quarantaine plagen.
- Een business plan wordt gemaakt voor de samenwerking in onderzoek op CATT en CT/röntgen onderzoek.
- Researchers visited IITE and discussed about the application of funds for collaborative research in 2014.

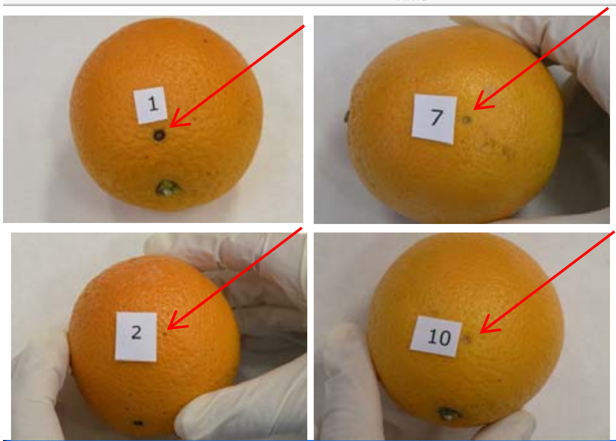
Trips Thailand
- Een bezoek door twee WUR onderzoekers aan diverse onderzoek instituten, inspectie centers en een aantal orchidee telers.
- Alternatieve duurzame oplossingen voor quarantaine plaag bestrijding (voornamelijk trips) gediscussieerd met de bedrijven en onderzoekers.
- Partijen uit Thailand hebben speciale interesse voor CATT behandeling en lure-and-kill techniek met biocontrol middelen.

Boorvliegen op mango

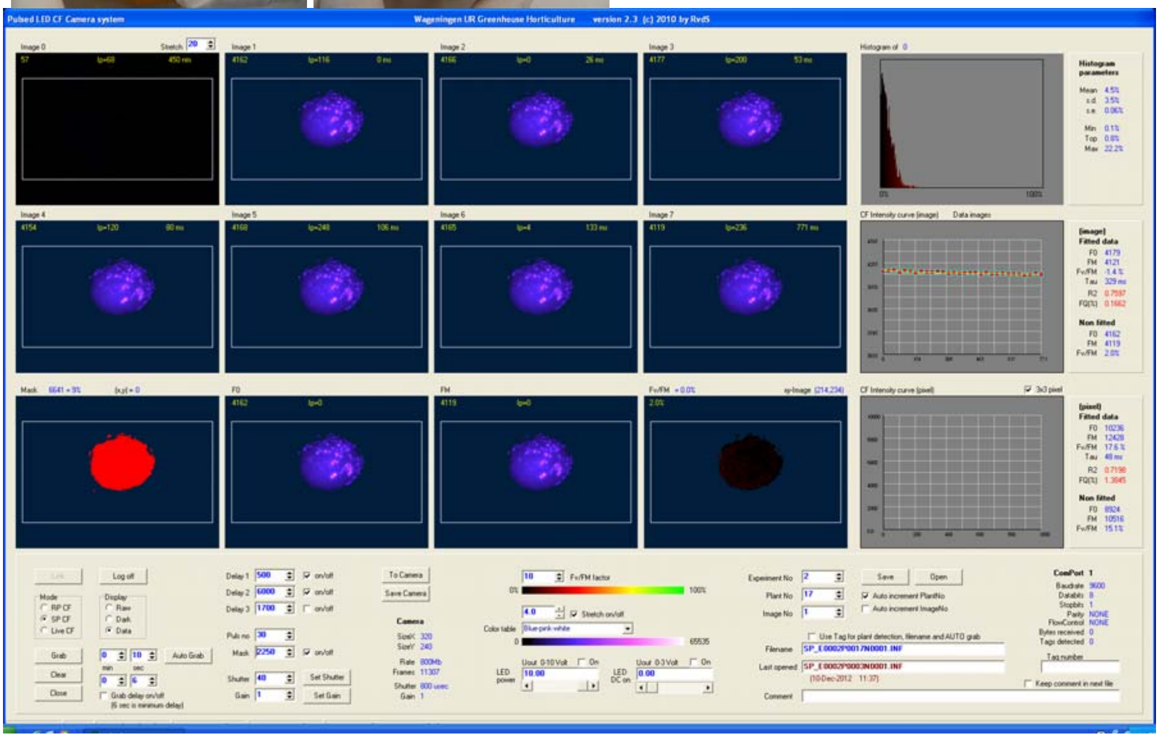
- Interview met KCB uitgevoerd de significantie van de boorvlieg probleem te verhelderen in diverse import producten.
- Een heldere beeld gekregen op import inspectie procedure (in land van oorsprong)
- Conclusie is dat een verbeterde certificatie systeem in de land van oorsprong zou niet helpen met de probleemoplossing.

Bijlage 1 Detectie van *G. citricarpa* van verschillend stadia met LAMP en imaging techniek.





1: Lesion nr. 1
2: Lesion nr. 2
3: Lesion nr.7
4: Lesion nr. 10
6: *Guignardia citricarpa* mycelium (pos control)
7: *Guignardia magniferae* mycelium (neg control)



Bijlage 2 Interview met KCB over boorvliegen in mango

Dik de Winter, Specialist Fytosanitaire Zaken

Bezoek Rayonkantoor Poeldijk, ABC Westland 108, na eerst telefonisch contact, gevolgd door e-mail contact:

Doel gesprek:

- Aanscherpen beeld importproblematiek boorvliegen in mango, zowel wat Q-organismen betreft als wat niet-Q organismen betreft.
    - Herkomsten
    - Vergelijkbare problematiek met andere producten? Tafeldruiven uit Brazilië en Chili bijvoorbeeld?
- Beeld van werkwijze importinspecties (en exportinspecties in landen van herkomst)
- Bespreken wat kansrijke oplossingsrichtingen zijn: certificeringssystemen in land van herkomst? Wat kunnen we leren van jullie ervaring? Andere oplossingsrichtingen.

Belang KCB:
- inzicht in risico-ontwikkeling. Zij zijn daar pro-actief mee bezig.
- minimaliseren impact fytosanitaire inspecties voor klant: hoe sneller definitieve uitslag, hoe liever. Zij verbreden graag hun mogelijkheden voor een snelle detectie op eigen locatie. Belang daarbij is dat het resultaat erkend wordt door de NVWA.
- Snelle identificatie is interessant voor KCB: sneller uitslag, minder lange vastleggingen, minder ‘terugkom bezoeken’ bij bedrijven. Zijn op de hoogte van werk WUR-PRI in FP7 aan snelle toetsen (‘faster than a coffee break’ Cor Schoen, Peter Bonants).

KCB: deed al langer kwaliteitsinspecties. Doet er sinds aantal jaren ook de fytosanitaire inspecties bij: efficientieslag en verlaging inspectiekosten voor klanten. KCB probeert tarieven in de hand te houden: tarieven al jaren niet verhoogd.

Boorvliegen in Mango:

2013: meer aantreffingen boorvliegen dan in 2012. 2013, rayon Zuid West: 30 vastleggingen boorvlieg wat inderdaad Q-boorvlieg bleek tot 15 november:”Bactrocera en Anasthrepha kwamen voor. houdt statistieken bij: aantreffingen en herkomsten, voor jaarlijkse rapportage.

2012: vaak boorvliegen aangetroffen in mango bij Cool Control (veelal C. Capitata, MedFly). In 2013 bij Mooy Logistics, Roveg, ADB (allen service verleners, dus niet dé belanghebbenden.

Herkomsten: meer aantreffingen bij ready to eat concept mango’s. Deze komen vooral uit West Afrika, per vliegtuig. Typisch voor deze mango’s uit West Afrika: kleine telers, verschillende telers per pallet, soms slechts enkele dozen per teler. Deze zijn rijper geoogst(?) en daarom meer kans op infectie door boorvlieg in land van herkomst en ontwikkeling symptomen door boorvlieglarve in het logistieke proces.

De bulk aan mango’s uit bijv. Brazilië komt van grote telers. Dit kunnen meerdere containers van dezelfde telers zijn voor één zending naar één importeur.

Andere gewassen met boorvliegen: ook Guave, Carambola (sterfruit), Pomelo (China, tevens ‘Ten Spot’, was eerder reden voor vasthouden partijen om te checken op ‘Black Spot’. Nu niet meer, maar NVWA wil wel monsters ontvangen om te monitoren of Black Spot voorkomt. Hete pepers: was niet inspectiewaardig voor Q-organismen. Bij controle bleek 8% besmet met boorvliegen. Wordt daarom nu wel een inspectiewaardig product.

Geen aantreffingen op tafeldruiven.

Meerwaarde certificering?

Meerwaarde certificering mango's irt boorvliegen:

- Niet direct aanleiding inspecties te wijzigen
- Pas als langere tijd minder aantreffingen voorkomen, kan intensiteit inspecties verlaagd worden. Dus na-ijl-effect als certificering minder besmettingen/infecties tot gevolg heeft.
- Wel direct kostenbesparing bedrijven bij minder aantreffingen.

Inspecties en Kosten

Werkwijze inspecties met als voorbeeld Mango – boorvliegen:

- per container een inspectie: aantal pallets worden in aparte ruimte klaargezet voor inspecties: vaste steekproefgrootte (aantal vruchten? of dozen?) per product.
- De inspecteur mag altijd zeggen dat ie alsnog product van een andere pallet wil controleren.
- beoordeling uitwendig, enkele worden doorgesneden, bij stipjes wordt voorzichtig gesneden om op inwendige symptomen te checken.
- check paspoortgegevens met gegevens op papier dat aan de pallets vast zit
- afkeur per partij. 1 partij = de hoeveelheid op het exportcertificaat/paspoort. Voor risicospreiding meestal 1 paspoort per container.
- Op paspoort mango's geen 'bijschrijvingen' met specifieke eisen kwaliteitscontrole in land van herkomst. Het is dus niet gedefinieerd hoe de exportcontrole in het land van herkomst plaatsvindt / uitgevoerd moet worden.
- bedrijf waar het product staat moet een assistent en beschikbaar hebben bij de keuringen. De assistent pakt de dozen met vruchten en wijst de weg, maar de inspecteur zegt welke dozen.
- Bij aantreffen: partij in quarantaine (aparte cel) tot definitieve uitslag.
- steeksproefgewijs controle of partij nog in quarantaine staat op het bedrijf.
- Binnen 4 weken moet eigenaar aangeven wat hij met partij van plan is. 2 mogelijkheden: 1: export naar buiten de EU. Bewijs moet geleverd worden dat partij daar daadwerkelijk aangekomen is. 2: vernietiging (verbranding)
- Bij aantreffen boorvliegen: Op kantoor 1^{e} lijn inspectie: check of het quarantaine is of niet. Maar NVWA moet ook nog controleren voordat partij vrijgegeven wordt. **Dit kost meestal 2-3 dagen.**
- KCB brengt kosten per minuut + voorrijkosten in rekening + eventuele analysekosten. Alles wordt doorberekend.

Importeurs versus Logistieke dienstverleners: verschil in belangen

Veel binnenkomende containers worden verwerkt door logistieke dienstverleners, zoals het bedrijf waar we meegekeken hebben bij een inspectie. Zij handelen niet in het fruit, maar doen opslag, herverdelen, faciliteren de keuringen en desgewenst ompakken/stickeren, etc.

Op basis van gesprek met De Winter: De logistieke serviceverleners hebben mogelijk niet veel nadeel van de Q-problematiek. Kosten worden doorberekend aan de klant. Ook het in quarantaine houden (extra cel in verhuur) hoeft financieel voor hen niet nadelig te zijn. Belang voor de importbedrijven zal dus groter zijn, dan belang voor serviceverleners.

Voor Q-organismen in meeste gevallen nog nacontroles door NVWA verplicht = extra tijd en kosten na 1e lijns controle bij KCB. Belang voor bedrijfsleven: meer erkenning van controles door KCB. KCB is daarom geinteresseerd in vergelijkend objectief onderzoek naar betrouwbaarheid snelle detectie.

Aantal bedrijven in NL die exporteren hebben goede ervaringen met pre-shipment inspecties: bijv. Japanse inspectiedienst die paprika's en bloembollen voor export keurt.: meer zekerheid dat product dat verzonden wordt, compleet en op de verwachte tijd bij de klant komt.

Andere hot-Q-issues:

Pepper weevil (risico bij import, o.a. uit Dominicaanse Republiek). vergelijkbare risico als wat we zien met Suzuki fruitvlieg: bedrijven die teelt en import combineren lijken hier een risicofactor. Paprikabedrijf besmet waarvan buurman hete pepers importeert.

Afrikaanse Fruitmot (AFM): issue in o.a. Pomelo's. Japan voert monitoring (op AFM?) uit rond teelt- en logistieke hotspots in NL.

Jaguar, Sven Thomas:
19-11-2013
Telefonisch contact. 0180-750526. sven.thomas@jaguartfc.nl, Quality manager
Prettig gesprek. Boorvlieg voor hen niet echt een issue. Mango doen ze niet in. Meer interesse in Black Spot, want groot in citrus. Gelinkt naar Peter Bonants. Sven was niet op de hoogte van het werk van Peter Bonants in BOCI. Als ik het goed begrepen heb is Sven's vrouw of haar familie verbonden aan / eigenaar van een teeltbedrijf in Brazilië. Sven heeft ook daar gewoond. Heeft ook contact gehad met Matthijs Montsma (WUR-FBR) over andere zaken.

> *Beste Peter,*
> *Voor het BOCI project had ik zojuist telefonisch contact met Sven Thomas, verantwoordelijk voor kwaliteitsmanagement bij fruithandelsbedrijf Jaguar. Boorvliegen is voor hen niet zo'n issue (zij importeren ook geen mango). Black spot wel. Zou je Sven Thomas een update willen sturen van wat je hebt lopen rond Black Spot? Sven gaf aan geïnteresseerd te zijn en is bereid mee te denken, en evt. mee te werken in pilots. Sven heeft goede contacten in Brazilië (daar ook gewoond).*
> *Sven Thomas is opvolger van Lieuwe Smit. Lieuwe heeft begin dit jaar deelgenomen aan een telefonisch overleg met enkele importbedrijven over Guignardia in citrus en boorvliegen in Mango. Lieuwe is opgevolgd door Sven Thomas.*
>
> *Bijgevoegd info van Sven Thomas over een middel om Black Spot te remmen.*

Staay Hispa, Lukas Meints

19-11-2013

Telefonisch contact. 0180-679100, Quality manager

Prettig gesprek, wel wat lastig te verstaan. Zijn standpunt toegelicht. Geen behoefte om betrokken te zijn bij vervolgacties. Informatie uit het project komt, indien relevant, via Frugi Venta wel zijn kant op. Verwijst voor betrekken importbedrijven naar Frugi Venta, werkgroep Fytosanitaire zaken.

- Ernst problematiek Q-organismen bij import valt voor hen mee:
  - probleem is voor hen beperkt. Kosten zijn voor exporterende partij. Het uitvallen van partijen geeft wel extra regelwerk, maar ervaart hij niet als problematisch. risico op uitval wordt soms al ingecalculeerd.

- Wel belang in verhoging kwaliteitsbewustzijn bij medewerkers in exporterende landen
  - Preventieve monitoring en maatregelen in de teelt
  - Stimulans moet uit het exporterende land zelf komen
    - Voorlichting en training
    - Hoe actie in gang zetten? Benader de organisaties voor exportbevordering in de exporterende landen

- Ziet niet veel kansen in certificering als stimulerings/of borgingsinstrument, omdat:
  - niet afdwingbaar, op vrijwillige basis: daarom bereik beperkt. pas echt waardevol als een hele sector het oppakt. Verwijst naar Black Spot in Zuid-Afrika als positief voorbeeld. *vergeten op door te vragen hoe dat opgepakt is. Wat was stimulerings / borgingsinstrument?*
  - certificering weinig garantie voor daadwerkelijke verhoging kwaliteitsbewustzijn. Risico van 'papieren tijger' zonder meerwaarde.
  - slechts klein deel (10%?)leveranciers échte ondernemers die willen investeren in verhoging kwaliteitsniveau
  - controles ivm met Q worden op landenniveau geregeld. Certificering van een aantal leveranciers zal niet snel gevolgen hebben voor keuringen (frequentie, kosten)
  - Werken niet met vaste leveranciers. Voor hen daarom minder interessant om met leveranciers te investeren in kwaliteit.
  - Als leverancier herhaaldelijk slechte partijen levert, doen ze vanzelf geen zaken meer met hen.

- Nationaal op te pakken (in Brazilië door Braziliaanse overheid)?
  - Bij een Q-issue in Nederland voor export ervaren dat een certificering/monitoring ook te hoogdrempelig kan zijn. Exporterende bedrijven stopten met export op het land waarvoor de extra eisen golden. Kosten waren hoog en nog groot risico dat partijen afgekeurd werden.
- Staay-Hispa heeft zelf geen fytosanitair specialisten in dienst.

Nature's Pride, Karel van der Linden / Martine Hosman 0174-525900

Bijlage 3 Student stage verslag

Lure and trap development for the citrus longhorned beetle *Anoplophora chinensis* Forster (Coleoptera: Cerambycidae: Lamiinae) by studying its chemical ecology

Name: Eline H. Verbon
Student number: 910329869020

Supervisors: Dr. Ir. A.J.M. Loomans (NVWA), Dr. Y.T. Qiu (WUR) and Dr. P. Lu (Beijing Forestry University)

Date: 30 January 2014

Joined internship between the Department of Entomology of Wageningen University (WUR) and the Laboratory of Wood Boring Insects of Beijing Forestry University.

1. Abstract

Anoplophora chinensis (Forster) is part of the family Cerambycidae, commonly known as longhorn beetles due to their characteristic large antennae. *A. chinensis* is native to China, Korea and Japan, but is regularly imported into nonnative territories, such as Europe, within living wood material. This poses a serious threat to European forests since *A. chinensis* infestation can lead to massive tree death. This is exemplified by the damage induced by an infestation of *A. chinensis* in Lombardy, Italy. After detection of the beetle in 2000, eradication programs were only set in motion in 2004. By now, the infestation has led to the death of thousands of trees and total eradication has not been achieved to this day. Clearly, early detection of this pest is of utmost importance.

Currently, the olfactory cues used by longhorned beetles to locate both host plants and potential mates are investigated as possible attractants in trap-lure combinations. If effective, these combinations might provide a means of rapid detection of invading longhorn beetles in nonnative territories. Although little is known about olfactory cues used by *A. chinensis*, several chemicals are known to attract its closest relatives *Anoplophora malasiaca* (Thomson), which has recently been synonymised with *A. chinensis*, and *Anoplophora glabripennis* (Motschulsky). *A. malasiaca* is attracted by the commercially available β-caryophyllene and α-humulene. These volatiles function both as long-distance indicators of host location and as short-distance indicators for males to locate female beetles. (-)-linalool and cis-3-hexen-1-ol are plant volatiles that function as long distance attractants for *A. glabripennis*. These volatiles are emitted by a large range of its host trees in the USA, but not by non-host species.

At Beijing Forestry University, the attractiveness of β-caryophyllene, α-humulene, (-)-linalool and cis-3-hexen-1-ol to *A. chinensis* was tested in Y-tube olfactometer assays. Test beetles were collected on Pingtan Island - Fuzhou, Fujian province, China. At this location, field trapping experiments were conducted with cross-vane panel traps. In the Y-tube assays 10^{-3} dilutions of α-humulene attracted females. Due to practical problems, the number of replicates was low in all experiments, with a maximum of 14 beetles per tested substance. In the field, none of the different mixtures of the four compounds resulted in any significant catches or bycatches.

Although no attractive effect of the tested chemicals was found in the field, there was an attractive effect of α-humulene in the Y-tube assay. Moreover, knowledge was gained on foodstuffs preferred by the beetles, on the evaporation rate of the tested compounds and on the large effect of the weather on beetle activity. This knowledge will be useful in future studies on *A. chinensis*, preferably with a higher number of beetles, more uniform living conditions throughout the experiments and trials with lures at times that they are known to have uniform evaporation rates.

2. Introduction

Anoplophora chinensis (Forster), commonly known as the citrus longhorned beetle (CLB) is native to China, Korea and Japan. *A. chinensis* is polyphagous; it has a broad host range, ranging from fruit and ornamental trees to conifers. Among these, the beetle specifically selects the healthy trees (Wang, 1998; Haack et al., 2010). While feeding on trees will not cause tree death, larval development within and adult emergence from trees may weaken trees to such an extent that death ensues (Hanks, 1999). *A. chinensis* does not only threaten trees in forests,shelterbelts and plantations in China, but also in Europe and America due to importation from East Asia within living plant material (Gaag et al., 2010; Haack et al., 2010; Hérard at al., 2006). To prevent *A. chinensis* establishment in Europe and the USA, early detection is of utmost importance. An adequate and intensive surveillance program is an important means for early detection of new *A. chinensis* populations. An efficient trapping system, using trap-lure combinations specifically designed for this species, could greatly enlarge early detection when *A. chinensis* densities are still low. This project evaluates the attractiveness of several chemicals to *A. chinensis*. The chemicals tested are known to attract *A. chinensis* [*as Anoplophora malasiaca* (Thomson)] and *Anoplophora glabripennis* (Motschulsky). The results of this study provide a first step in the development of the optimal trap-lure combination for the detection of *A. chinensis*.

2.1 Taxonomy and appearance

Anoplophora chinensis is part of the family Cerambycidae (Coleoptera), which includes about 35.000 species, divided into nine subfamilies. *A. chinensis* is part of the genus *Anoplophora* Hope and has recently been synonymised with *Anoplophora malasiaca* (Thomson) (Lingafelter & Hoebeke, 2002). *A. chinensis* is generally 17-40mm long and has white patches on its elytra. The base of the elytra is covered with short processes, so-called granulae. Two stout spines are present on either side of the beetle's pronotum. The antennae are composed of 11 segments. The basal part of each segment is white, whereas the distal part is black (figure 1). The female is generally the larger sex and often has the tip of her abdomen exposed. The abdomen of males is entirely covered by their elytra (Lieu, 1945).

The closest relative of *A. chinensis* is *Anoplophora glabripennis* (Motschulsky) (ALB), also cited under its synonym *Anoplophora nobilis* (Ganglbauer) (Lingafelter & Hoebeke, 2002). *Anoplophora glabripennis* has an appearance that is very similar to *A. chinensis*, but it lacks granulae, has yellow spots on its elytra and has black-and-blue antennae (figure 1) (Haack et al., 2010). The antennae are the most reliable exterior indicator of sex in *A. glabripennis*. Generally, *A. glabripennis* males' antennae are close to

Fig. 1 A female *Anoplophora chinensis* (right, 2.9cm from start of antennae to base of elytra) and a female of its closest relative *Anoplophora glabripennis* (left, 3.1cm). *A. chinensis* can be recognized by the white spots on its elytra, the tubercles on the base of its elytra and its black-and-white antennae. *Anoplophora glabripennis* has smooth elytra with yellow spots and black-and-blue antennae. Beetles provided by Eline Verbon. Photo by Lily Ren.

two times body length, whereas the antennae of females are generally less than 1.5 times body length. In addition, the ventral side of the last antennomere (antennal segment) is partly white in females, whereas is it usually fully black in males (Hajek et al., 2004). Conclusive sexing of the beetles can only be performed after dissection (Hajek et al., 2004).

2.2 Life cycle

A. chinensis oviposits in both old and young healthy trees (Hanks, 1999). Oviposition generally takes place on the lower trunk, root collar region and exposed roots (Haack et al., 2010), although oviposition sites have also been found higher up in trees (Gaag et al., 2010). Female *A. chinensis* chew a slit in the bark to form an oviposition site. A single egg is subsequently placed inside. The eggs hatch approximately 1-2 weeks later (Haack et al., 2010). Larvae only spend a brief period under the bark and soon tunnel their way into the wood (Hanks, 1999), while expelling frass at the bark. *A. chinensis* generally overwinters as a larva and pupates 1-2 years after oviposition, depending on the climate. In Fujian, China, the beetles normally pupate in late spring or early summer, with the peak in May to July. Adults emerge from circular holes that generally have a diameter of 10-15mm. After emergence, adults undergo 10-15 days of maturation feeding on tree twigs to become sexually mature (Haack et al., 2010). After maturation and copulation, females can lay 90-320 eggs during their lifespan of 3.5 months (Peverieri & Roversi, 2010).

2.3 Mate location in longhorned beetles (Cerambycidae)

In order to reproduce, *A. chinensis* needs to locate a mate of the other sex. Although little research has been done on mate location in *A. chinensis*, a general pattern of mate location is starting to emerge for all Cerambycidae. First, beetles of both sexes are attracted from their natal tree to another host plant, so-called long-distance attraction. Long distance attraction is generally mediated by host plant emitted volatiles. Subsequently, either one or both of the sexes secrete pheromones, which function as short distance attractants to the opposite sex. Finally, males locate the females via antennal contact (Ginzel & Hanks, 2005). Although different subfamilies use different pheromones in mate attraction, the pheromone structures are often highly conserved among closely related species of Cerambycidae (Teale et al., 2011).

Long-distance attraction was first recorded in 1996. Hanks et al. (1996) studied host location by *Phoracantha semipunctata* F., the eucalyptus longhorned borer. The time of arrival of these beetles on a host plant is sex-independent, indicating that neither of the sexes is dependent on the other to find the plant. Moreover, neither sex responds to the opposite sex when placed downstream of it in a wind-tunnel (Hanks et al., 1996). Thus, long distance host location is not mediated by beetle-produced volatiles, but by volatiles emitted by the host plant itself. Long distance attractants from host-plant volatiles for *Anoplophora glabripennis* were first isolated and identified by Li et al. (2003) from *Acer negundo* L.. They showed that cis-3-hexen-1-ol and a mixture of 1-butanol, 1-pentanol and 2-pentanol attracted *A. glabripennis* in the field. A subsequent study on a large range of host trees in the USA uncovered (Z)-3-hexen-1-ol, (E)-caryophyllene, delta-3-carene, (-)-linalool, linalool oxide and camphene as volatile compounds common in host trees and absent in non-hosts (Wickham & Teale, as cited in Nehme et al., 2009). Of these chemicals, (Z)-3-hexen-1-ol and (-)-linalool significantly increased short-range attractiveness of lures designed to attract *A. glabripennis*. In addition, both substances contribute to attractiveness of lures in the field (Nehme et al., 2009). Although aggregation of longhorn beetles on host plants is generally thought to occur in response to host plant volatiles, both *Neoclytus acuminatus acuminatus* Fabricius, commonly called the red-headed ash borer, and *Neoclytus mucronatus mucronatus* F. males have been shown to excrete pheromones that are attractant to both sexes in field conditions. Total-ion chromatography uncovered (2S*,3S*)-hexanediol as the attractant excreted by *N. a. acuminatus* males and (R)-3-hydroxyhexan-2-one by *N. m. mucronatus* males (Lacey et al., 2004; Lacey et al., 2007).

Recently, Wickham et al. (2012) showed that virgin *A. glabripennis* female cuticular extracts enhance attraction of *A. glabripennis* males to host kairomones in the field. Thus, females might be attracted to host trees by host kairomones and subsequently produce volatiles that function together with host kairomones as long distance attractants for *A. glabripennis* males (Wickham et al., 2012). Interestingly, the composition of female cuticular extracts are age- and mating status dependent. Virgin *A. glabripennis* beetles produce pheromones in maximum abundance until mating, after which the production is immediately shut off (Wickham & Teale, 2009). Likewise, male *A. glabripennis* excrete 4-(n-heptyloxy)butan-1-ol and 4-(n-heptyloxy)butanal (Zhang et al., 2002), which attract female beetles in the field (Nehme et al., 2009). However, females generally walk to these traps, instead of flying to them. Thus, this is probably not a long-range, but rather a short range attractant (Wickham et al., 2012). *A. malasiaca* seems to use volatiles originating from its host tree *Citrus unshiu* as both long-range and short-range attractants. Long-range attraction is probably directed by volatiles being directly emitted from the tree. Short-range attraction, on the other hand, is probably mediated by release of the same volatiles from the elytra of female beetles. Both host tree emitted volatiles and those released from the elytra are composed of five major sesquiterpenes, namely β-elemene, β-caryophyllene, α-humulene, α-farnesene and an unidentified one, together with several unidentified minor sesquiterpenes. The cuticular extract of beetles fed on an artificial diet do not contain these volatiles, making it likely that the volatiles are either taken up via the air or via the digestion pathway during feeding. In short-range laboratory experiments with the commercially available α-humulene and β-caryophyllene, males were attracted to both single compounds. Moreover, single α-humulene and a mixture of α-humulene and β-caryophyllene evoked a response almost equal to the crude female cuticular extract. Field experiments show attraction of male beetles to the sesquiterpenes. This attraction is probably of the long-range type, as indicated by beetles walking on the ground surface and flying for longer distances (Yasui et al., 2007a).

In 1996, Fukaya et al. first identified a contact sex pheromone in the cerambycid species *Psacothea hilaris* (Pascoe), or the yellow-spotted longicorn beetle. Males of this species will dash to females once in their proximity and execute copulatory behaviours after touching the female. The latter response was shown to be caused by the cuticular hydrocarbon (Z)-21-methyt-8-pentatriacontene, the major component of the crude female elytra extract (Fukaya et al., 1996). Similarly, close proximity of the opposite sexes of *Megacyllene robiniae* (Forster) beetles does not lead to mating unless males touch the females with their antennae. Recognition is mediated by molecules on the elytra of the females, since washed females do not elicit a response in the males (Ginzel & Hanks, 2003). The molecule responsible for this reaction is Z9:C25, the most abundant hydrocarbon on the surface of the cuticular wax layer of females (Ginzel et al., 2003). Likewise, the presence of sex pheromones in the female cuticular extract of *A. chinensis* was shown in 1998 by Wang, although the identity of the chemicals was not determined (Wang, 1998). In the conspecific *A. malasiaca*, female cuticular contact sex pheromones consist of two fractions: one fraction consisting of eight major hydrocarbons (Fukaya et al., 2000) and the other fraction consisting of polar compounds, containing at least five ketone compounds (Yasui et al., 2003) and three lactones (Yasui et al., 2007b).

Visual cues are also involved in mate location (Fukaya et al., 2005). *A. malasiaca* females are attracted more to black rods than to white rods, possibly because the beetles are mostly black themselves. Visual cues only influence mate location when accompanied by olfactory cues. Thus, visual cues probably function only as enhancers of olfactory cues (Fukaya et al., 2005).

2.4 Anoplophora chinensis in Europe and North America

As mentioned, larval development of *A. chinensis* within host trees can lead to tree death. To prevent establishment of this pest and the associated costs (Haack et al., 2010) *A. chinensis* has been assigned the status of quarantine pest in Europe and North America (EPPO 2013; Gaag et al., 2010). In the EU, emergency measures against *A. chinensis* have been in place since November 2008 (EC 2008; Gaag et al., 2010) because of increasing numbers of intercepted *A. chinensis*. Most of these beetles come from China (85%), while some originate from Japan and Korea (Haack et al., 2010).

A. chinensis was first intercepted outside its native range in the Netherlands in 1980. From then on, the amount of interceptions increased until a peak was reached in 2008. This peak was possibly due to an increase of live import from Asia or an increase of *A. chinensis* near to nurseries in Asia exporting trees to Western countries (Haack et al., 2010).The first established *A. chinensis* population in nonnative territory was discovered in Lombardy, Italy, in 2000, probably years after the original introduction of the beetle. Eradication measures were initiated in 2004. By 2005, the beetles had spread among 16 municipalities, while hundreds of trees had been destroyed or cut down in an attempt to eradicate the beetle (Hérard et al., 2006). By 2009, 10,000 trees of more than 20 species had been attacked in North Italy. Efforts are still ongoing to eradicate the beetle (Maspero et al., 2012). This infestation spread awareness of the necessity to rapidly detect and respond to *A. chinensis* infestations. Subsequent infestations in France (2003), Croatia (2007) and the Netherlands (2007 and 2009) were detected sooner and eradication measures started up more rapidly. These could therefore be reacted upon more efficiently. The infestation in France has been officially eradicated since 2006 (Gaag et al., 2010), those in the Netherlands, in Boskoop, and Croatia since 2011 and 2013 (NVWA, personal correspondence). No established population of *A. chinensis* has been found in the USA and Canada until now. In 2001, however, three adult beetles were captured at a bonsai nursery in Washington State. Subsequent inspection of the Korean bonsai maple trees revealed eight exit holes. In reaction, Washington State implemented an eradication program that lasted until 2007, cost US$2.2 million and involved cutting down more than 1000 trees. In 2007, the program was ended because no beetles were ever found outside of the nursery (Haack et al., 2010).

In Europe, all woody deciduous plants can be considered a potential host for *A. chinensis*, while conifers most probably do not serve as a host plant. Although the lifecycle of *A. chinensis* in Southern Europe is comparable to the lifecycle in its native habitat, the lifecycle in Northern Europe seems to be longer, possibly ranging up to four years in the larval stage, due to the cooler climate. In addition, reproduction rate seems to decrease in lower temperatures. Because infestations are thus highly variable, it is difficult to set standard eradication measures (Gaag et al., 2010). Generally, infested trees are cut down and studied for the presence of larvae or eggs upon detection of *A. chinensis*. In addition, the surrounding area is thoroughly examined for exterior symptoms of the presence of adult and other stages of *A. chinensis* infestations. The former is indicated by typical feeding scars on host trees of *A. chinensis* (figure 2), whereas the latter is shown by the presence of oviposition slits, frass expulsion, resulting from larval boring, and emergence holes. The absence of exterior infection signs does not conclusively rule out the presence of an infestation inside the tree. Therefore, apparently healthy trees in the close surrounding of infested trees are sometimes cut down as a precautionary measure. Surveys in the surrounding area are generally performed for several years (Gaag et al., 2010). In the case of the established infestation in Italy, the accidentally introduced egg parasitoid *Aprostocetus anoplophorae* has been used as an efficient natural enemy of *A. chinensis*. Although release of the parasitoid will not lead to total eradication, it will prevent *A. chinensis* from spreading further while effective eradication measures are developed (Maspero et al., 2012).

Once an infestation is established, eradication efforts are time consuming and expensive. Therefore, early detection of *A. chinensis* is of utmost importance. The potential of both host plant volatiles and beetle emitted pheromones as cerambycid attractants is now recognised (reviewed by Allison et al., 2004) and many studies are carried out on developing effective trap and lure combinations with these substances (for examples, see Nakamuta et al., 1997; Nehme et al., 2009; Nehme et al., 2010; Reddy et al., 2005; Wickham et al., 2012; Yasui et al., 2007a).

Fig. 2 Feeding marks by *A. chinensis* on *Melia azedarach* L., a feeding tree of *A. chinensis* (left) and on its host tree *Casuarina equisetifolia* L. (right). Photos by Eline Verbon.

This project studies whether *A. chinensis* is attracted to β-caryophyllene, α-humulene, linalool and cis-3-hexen-1-ol. As described above, the first two substances are known to attract *A. malasiaca* both from long and short distances. Linalool and cis-3-hexen-1-ol are emitted by many host trees of *A. glabripennis* and attract this species both in short-range and long-range experiments. Because of the close relationship of *A. chinensis* with both these species, it seems plausible to suggest these substances are possible attractants for *A. chinensis* as well. The substances are tested both in laboratory assays, to determine short-range attraction, and in field experiments, to determine possible long-range attraction. In the future, the results of this and further experiments will hopefully result in the development of an effective trap-lure combination for *A. chinensis*, thereby increasing detection rate and preventing establishment of this species outside its native habitat.

3. Materials and methods

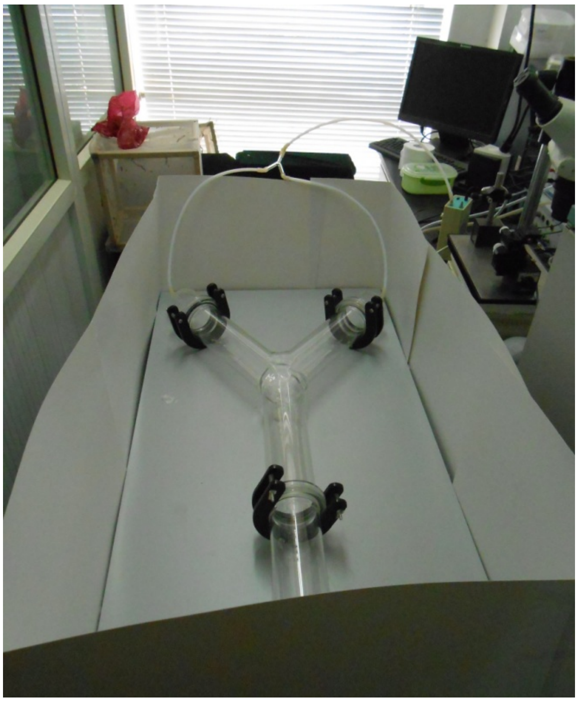
3.1 Y-tube olfactometer experiment

Y-tube olfactometer experiments were conducted between 8 am and 6 pm from June 12th to June 28th 2013 in a laboratory of Beijing Forestry University campus, Beijing, China. In the Y-tube olfactometer, male and female *A. chinensis* adults were exposed to different concentrations and mixtures of four commercially available chemicals: β-caryophyllene, α-humulene, linalool and cis-3-hexen-1-ol. All substances were offered against a control. The response of the beetles was recorded and analyzed to determine whether these chemicals are attractive or repellent to *A. chinensis*.

3.1.1 Experimental set-up

The Y-tube olfactometer was manufactured at Xinweier Glass Instrument Ltd., Beijing, China. The Y-tube olfactometer consists of four parts: a Y-tube and three glass extensions. The extensions close each of the Y-tube's openings. The whole structure is made of glass, with an inner diameter of 7 cm. The main arm of the Y-tube measures 34 cm, the two arms 28 cm each. The arms form an angle of 75°. Septa filled with either a test or a control compound are secured at the end of each arm on white, non-smelling clay within the 10 cm long glass extensions (figure 3). The glass extensions are attached to the arms with aluminum clamps to make the connection airtight. Air is pumped into each of the extensions via plastic tubes of 120 cm long and flows into the Y-tube along the test odours. Air leaves the Y-tube via the apex of the third glass extension at the base, which measures 29 cm. This extension is fastened to the Y- bottom of the main arm of the tube after a beetle is released into the Y-tube (figure 3).

During the first day of experiments and the first four trials on the second day airflow was set at 3L/min. During the rest of the trails air flow was set at 4L/min because beetles seemed unresponsive at first. The Y-tube olfactometer was placed horizontally on a table under a 14W LED light. White cardboard was positioned around the table to ensure equal light and symmetric background on both sides of the Y-tube (figure 3). The Y-tube was disconnected and cleaned with a cotton ball dipped in pure ethanol (Beijing Chemical Works, China) after each trial. The base glass extension at the bottom of the Y-tube and the glass extension(s) at the end of the arm(s) that had contained a volatile and / or a beetle at some point during the previous trial were cleaned similarly. At the end of testing each set of different volatiles all parts of the Y-tube olfactometer were cleaned with pure ethanol. At the end of the day, the table was also cleaned with pure ethanol.



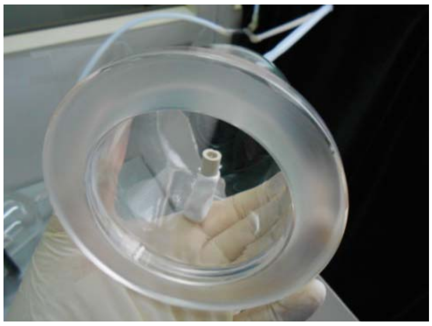


Fig. 3 The experimental set-up of the Y-tube olfactometer experiment. Left) Air is blown from the top of the arms into the Y-tube olfactometer by a pump. Testing odours are placed in the glass extensions at the end of each arm, thereby allowing the volatiles to be carried into the Y-tube along with the airflow generated by the pump. Beetles are released at the bottom of the main arm of the Y-tube. Afterwards, a longer glass extension can be connected to the Y-tube at this position. Above) the lures were placed in a glass extensions on top of molded white clay.

Each beetle was allowed to walk into the main arm of the tube. If the beetle did not move, it was lightly pushed with branches from its container. Entrance into the main arm was taken as the start time of the experiment. The experiment was ended when 1) one hour had passed, 2) when the beetle had remained in the main arm for more than half an hour, or 3) when it had passed the black connector at the end of either one of the two arms. A ‘no response’ was recorded in case of the first two experimental endings. In the latter case a response was recorded; either treatment or control. In total, we conducted 3 series of experiments. The schedule set up tested each of four chemical dilutions on twelve beetles in the first experiment. The schedule of the second and third experiment tested each of five chemical dilutions, one of which consisted of only the solvent, on fourteen beetles.

3.1.2 Preparation of the test compounds

β-caryophyllene (Sigma, USA; CAS: 87-44-5), α-humulene (Aldrich, USA; CAS: 6753-98-6), linalool (Aldrich, USA; CAS: 126-91-0) and cis-3-hexen-1-ol (Tokyo Chemical Industry, Japan) were in the Y-tube olfactometer experiments. These were dissolved into paraffin liquid (Xi Long Chemical Industry Co. Ltd., China) to 10^{-1} and 10^{-3} ppm. Dilutions of 10^{-1}, 10^{-3} and 10^{-5} are generally used in Y-tube olfactometer experiments with longhorned beetles (Lily Ren, personal communication). The middle concentration of 10^{-3} was tested first. Because of little response from the beetles, 10^{-1} was used for the following assay. Chemicals were loaded onto rubber septa (Beijing Ge Rui Bi Yuan Technology Co. Ltd.), which were cleaned by placing them in successively fresh hexane, C_6H_{14} (Beijing Chemical Works, China; Sinopharm Chemical Reagent Co. Ltd, China) twice for 3h, methanol (Beijing Chemical Works, China) for 3h and hexane for 23h. Afterwards, the hexane was removed and the septa were left to dry in the air. The dilutions were stored at -20°C after loading until further use.

In the first and second experiment, each septum contained 100 mg of one of the 10^{-3} dilutions or the 10^{-1} dilutions, respectively. In both experiments, the pure solvent was used as the control, also loaded with 100 mg per rubber septum. In the first experiment, loaded septa were left at room temperature for several hours before they were stored at -20° C until further use. The septa loaded with 10^{-1} dilutions were immediately stored at -20° until use. For the third and last experiment, the following four mixtures were loaded on the rubber septa: 1) β-caryophyllene and α-humulene, 2) linalool and cis-3-hexen-1-ol, 3) β-caryophyllene, α-humulene and linalool and 4) a mixture of all four volatiles. All mixtures were made from the 10^{-3} dilutions of the individual volatiles. The volatiles within one mixture were present in equal quantities and were thoroughly mixed by pipetting up and down several times. As in the first two experiments, the final weight of each septum was 100 mg and paraffin liquid was used as the control. The septa were left in the air for two hours after loading before being stored at -20° C.

3.1.2 Collection of the beetles of the first experiment

A. chinensis beetles were collected in farm and plantation areas in Li Shui village, Pingtan county, Fu Zhou city, Fu Jian province, China on June 7th and 8th. Beetles were captured either on China-berry, *Melia azedarach* L. (Meliaceae) or ”mu ma huang”, *Casuarina equisetifolia* L. (Casuarinaceae). Upon capture, beetles were immediately put in separate soft plastic boxes with sufficient air holes and supplied with *Melia azedarach* branches. Beetles were strong and active, as indicated by the escape of several of them by gnawing through the plastic of the boxes (figure 4). Beetles were put in boxes made from hard plastic (10 cm * 7,5 cm * 5 cm) on June 9th. Within these boxes, they were transported from Fujian to Beijing on the night train from June 9th to June 10th. From there, they were transported by car to Beijing Forestry University.

3.1.3 Food supply and gender identification

Fig. 4 *A. chinensis* in its successive containers. Above) An *A. chinensis* beetle in a soft plastic box, with a hole it gnawed through the plastic. Right) *A. chinensis* in hard plastic boxes within a closet placed in a courtyard at Beijing Forestry University, China. A plastic cover was used to protect the beetles' cages against the rain. Beetles were kept in a minimum number of layers on top of each other.

Fourty beetles arrived at Beijing Forestry University healthy and intact, i.e. without broken antennae or legs. In the laboratory beetles were placed on shelves within a cupboard, situated within a courtyard (figure 4) at ambient temperature. In the afternoon of June 11th, these beetles were supplied with fresh branches and leaves of Salix babylonica L. (Salicaceae) that had been collected the evening before on the campus of Beijing Forestry University. A. chinensis has been shown to be able to complete development on this species (Lingafelter and Hoebeke (2002), Haack et al., 2010). The branches and leaves had been washed and left to soak in water overnight to remove any possible pesticides present. All beetles seemed healthy and strong. That evening, the beetles' gender was identified by examining the ventral side of the last antennomere (antennal segment), which is partly white in females, whereas is it usually fully black in males. After the experiments, gender was determined conclusively by dissection. Six active male and six active female beetles were chosen to take part in the first experiment (figure 5).

The beetles did not eat from the willow branches and were therefore again supplied with *Melia azedarach* branches taken from Fujian on June 12th. These branches had partly dried out by then. In the night of June 13th-14th all beetles had become very weak and one experimental beetle died. During the day, another beetle died. The following day, June 14th, the beetles were given cotton balls dipped in honey water. In addition, a pail of water was placed on the lowest shelf in the cupboard and the cupboard was partly closed to increase moisture content within the closet. The following night, two more experimental beetles died. In the morning, all surviving beetles received *Acer truncatum* branches collected at the campus of Beijing Forestry University. These were washed and soaked overnight. Beetles which had little honey water left, were supplied with fresh honey water. At this point, the beetles were too weak to carry out further Y-tube trials.

3.1.4 The first Y-tube experiment: 10^{3} volatile dilutions against a control

The cages of the six male and female beetles selected for the first experiment were tagged with the number 1 through 12, in random order. During each day, three sets of four trials were executed. During each set of four trials, each volatile, i.e. β-caryophyllene, α-humulene, linalool and cis-3-hexen-1-ol, each in a dilution of 10^{-1}, was tested once. The volatiles within a set were tested in random order. Moreover, each volatile was assigned six times to the right and six times to the left arm randomly over the whole experiment. Each beetle was tested once per day. Over the four days that were scheduled for the experiment, all volatiles would be tested by each beetle once. The schedule was set up as described in appendix 1. During each experimental day, one septum was used per volatile. Similarly, one control septum was used for the whole day. Thus, each volatile was used three times and each control

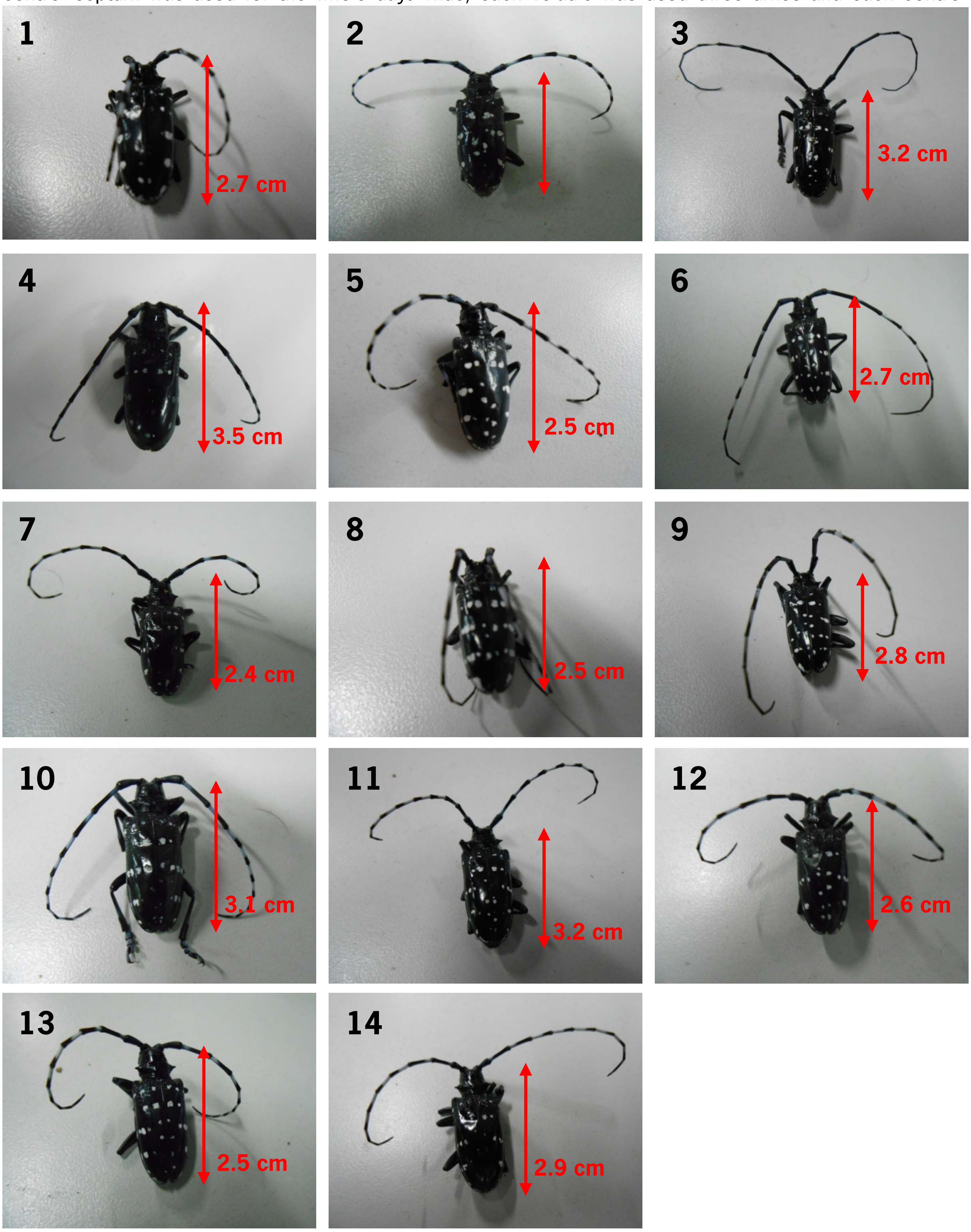


Fig. 5 The 14 beetles used in the first experiment, with the date of death between brackets. Beetle 1 (15-06), 2 (27-06), 4 (killed 02-07, was very weak at that point), 5 (14-06), 7 (17-06), 10 (18-06), 11 (19-06), 12 (21-06), 13 (16-06) and 14 (22-06) were identified as female after dissection. Beetle 3 (15-06), 6 (15-06), 8 (16-06) and 9 (14-06) were identified as male. Beetle 4, 10 and 14 had been incorrectly identified as males before dissection. Photos by Eline Verbon.

septum was used 12 times. During the third experimental day, two beetles had died and where replaced by other beetles (figure 5). On the fourth day, the first beetle put into the Y-tube did not move at all. Almost all other beetles were also too weak to carry out further Y-tube experiments. Therefore, no results were collected this day and only nine beetles were tested per volatile. The 12 individual beetles were tested once on each of three consecutive days (appendix 2) and stored and fed as detailed in section 3.1.3.

3.1.5 Collection and storage of the beetles of the second and third experiment

On June 16th and 17th a second batch of beetles was collected at the same site as the first batch in Pingtan, Fujian, China. These beetles were put in empty plastic drink bottles with sufficient air holes and supplied with *Melia azedarach* L. branches immediately after collection. After transport to Fuzhou, the beetles and branches were placed in the hard plastic boxes as described above. In the night of June 18 - 19, they were transported to Beijing by train. Like the previous batch, the beetles were placed in a cupboard within a courtyard on arrival at Beijing Forestry University (figure 4). Water buckets were placed on every shelf and the cupboard largely closed to increase moisture content in the cupboard as compared to the surroundings. The same evening, the beetles' gender was identified by examining the ventral side of the last antennomere (antennal segment), which is partly white in females, whereas is it usually fully black in males. The gender of the beetles was double-checked by examining whether the abdomen was exposed, as it usually is in females, or unexposed, as in males. Only beetles for which both characteristics indicated the same sex were used for experimental purposes. After the experiments, gender was determined conclusively by dissection. Between death and dissection, beetles were kept in 75% ethanol.

From June 20 to June 29, all beetles received some drops of water on the lids of their cage every morning around 8 am. In the evening of June 20, they were supplied with new *Melia azedarach* L. branches. These branches had been brought from Fujian and stored in the fridge. In addition, they received a few drops of water inside their cages. On June 22th, the beetles received both *Acer truncatum* and *Melia azedarach* L. branches. From then on, the cages of the beetles were emptied and the beetles supplied with new *Acer truncatum* branches every two days, by then the *Melia azedarach* L. branches had dried out.

3.1.6 The second Y-tube experiment: 10^1 volatile dilutions against a control

The second experiment was performed from June 20th to June 25th (appendix 3 and 4). Eight active females and seven active males were selected for participation. Because of the set-up of the experiment, five beetles (6, 8, 10, 14 and 15) were not used during the fifth day. To ensure an equal number of males and females tested for each volatile, these numbers were used to tag only female beetles. The rest of the cages were tagged randomly. During each day, three sets of five trials were executed. As in the previous experiment, each volatile, i.e. β-caryophyllene, α-humulene, linalool and cis-3-hexen-1-ol, was tested once per set of trials. A fifth septum filled with pure paraffin liquid, like the control, was added as a fifth condition. This extra control septum was designed to test the symmetry of the Y-tube. The four volatiles and the control within each set were tested in random order and each volatile was assigned randomly to be presented seven times in both the left and the right arm of the Y-tube. Each beetle was tested once per day. Over the five days that were scheduled for the experiment, all volatiles were tested by 14 of the 15 beetles: seven males and seven females. In between tests individual beetles were stored in the cupboard and fed as described in section 3.1.5.

Volatiles and controls were replaced after each experimental day.

3.1.7 The third Y-tube experiment: 10^3 volatile mixtures against a control

The third and final Y-tube experiment was commenced on June 27th. For this experiment, the same 15 beetles as used in experiment two were used. A new schedule was created for the execution of this experiment exactly as for the second experiment (appendix 3). Again, three sets of five trials were performed each day (like in appendix 4). In this case, four different mixtures and one control were tested against a control. The four mixtures and the control within each set were tested in random order and each volatile was assigned randomly to be presented seven times in both the left and the right arm of the Y-tube. Each beetle was tested once per day. Over the five days that were scheduled for the experiment, all volatiles would be tested by 14 of the 15 beetles: seven males and seven females.

For each trial, a new control and a new volatile mixture were taken directly from the -20° C freezer. On the second day, new controls were made for the remaining days of the experiment because they had run out.

On the fourth day of the experiment, after heavy rain during the night, the temperature in Beijing had dropped to approximately 25°C at midday. The following few days the temperature remained low and the skies cloudy. Under these conditions, the beetles became inactive, preventing performing any further Y-tube experiments. On July 2nd all beetles were stored in 75% alcohol. Beetle number 14 (June 27nd) and number 8 (July 2nd) had already died by then.

3.1.8 Evaporation rate measurement
Cumulative weight loss of the volatiles within the septa was used as a measure for evaporation rate. To determine weight loss, all septa were weighed after each trial throughout the second day of the experiment. After the end of the Y-tube olfactometer assays, two extra detailed evaporation curves were made. Both measured evaporation rate of 10^{-1} dilutions of the single compounds about once per hour throughout approximately ten hours. The first evaporation curve measured evaporation of chemicals from loaded septa of which neither the dilutions, nor the loaded septa had been frozen. The second evaporation curve measured evaporation of chemicals from loaded septa in which only the dilutions had been frozen.

3.1.9 Statistical analysis
Results were analysed with chi-squared tests. *3.2 Field experiment*
Thirty-five traps were set up in Pingtan, an island in front of the coast of Fuzhou, Fujian province, China. Different mixtures of β-caryophyllene, α-humulene, linalool and cis-3-hexen-1-ol were placed within the traps. The number of beetles and the number of *A. chinensis* caught were recorded per trap.

3.2.1 Preparation of the chemicals
β-caryophyllene (Sigma, USA; CAS: 87-44-5), α-humulene (Aldrich, USA; TCI, China, CAS: 6753-98-6), linalool (Aldrich, USA; CAS: 126-91-0) and cis-3-hexen-1-ol (Tokyo Chemical Industry, Japan) were used in their pure form in the field experiment. Septa were cleaned as described previously.

3.2.2 Setting up the traps
Chemicals and cleaned septa were taken from Beijing to Fujian (China) by plane on July 3th. On July 4th, 35 cross-vane panel traps were set up in seven rows of five traps. The rows were placed perpendicular to the direction of the wind. Each trap was hung from a branch of a tree close to the stem with the septum at a height of approximately 1.5 meters from the ground (see figure 6). Traps within a row were placed at approximately 30 meters from each other. Four rows were placed in a *Melia azedarach* L. tree plantation area, at 25.41413° N and 119.75199° E (like in figure 6). The other three rows of traps were placed in an area with multiple different tree species at 25.45002° N and 119.78180° E. Traps in the latters rows were hung from *Melia azedarach* L. All traps were placed between 14 and 19 meters above sea level.

The five traps in one row each contained one of the following mixtures: 1) α-humulene and linalool, 2) α-humulene, linalool and β-caryophyllene, 3) α-humulene, linalool and cis-3-hexen-1-ol, 4) humulene, linalool, β-caryophyllene and cis-3-hexen-1-ol and 5) control, i.e. no chemicals, in a random order. Mixtures consisted of pure chemicals, mixed to a total of 200µl per septum. One septum was placed in each trap. The collection bottles of two rows were supplied with sand mixed with the insecticide DDVP. The insecticide was added to prevent caught beetles from leaving the collection bottles or fighting inside the bottle with

Fig.6 The cross-van panel traps in the field. Beetles fly against the cross-vanes and fall into the collection bottle (white). Left) Traps were hung from branches close to the stem of the tree. Right) Lures were placed in the middle of the trap. While putting up the traps, care was taken to ensure a height of the lures of approximately 1.5m. Photos by Eline Verbon.

other caught beetles. However, the smell of the insecticide was very strong and we feared for it interfering with the smell of the mixtures. Thus, this was not added to any of the other collection bottles.

3.2.3 Recording trap catches and replacing the septa

Collection bottles were checked for and emptied of caught animals on July 5th and July 6th. The species identity of all animals caught was recorded. On July 21th, this procedure was repeated. In addition, the volatile mixtures in the five replicates without insecticide were replaced by new mixtures. These traps were supplied with the same mixture as before, but this time two septa containing 200µl instead of one septum was placed in each trap. The volatile mixtures in the other two rows of traps were not replaced, to try to determine when the volatile mixtures stop attracting insects. July 28th the traps were checked a final time and taken down. Presence or activity of adult beetles on the trees with the traps or in the near surroundings were not recorded.

4. Results

4.1 Y-tube olfactometer experiment

Three Y-tube olfactometer experiments were carried out using four commercially available volatile attractants: β-caryophyllene, α-humulene, linalool and cis-3-hexen-1-ol. The first and second experiment exposed adult *A. chinensis* beetles to the single compounds in 10^{-3} and 10^{-1} dilutions against the pure solvent. The third experiment tested mixtures of the compounds against the pure solvent. Twelve beetles, six defined as female after exterior examination and six defined as male, were originally chosen to participate in the first experiment. Two of these died before the experiment ended and were replaced by other beetles. Each beetle was tested once per day, with a different test substance each time. Thus, over four days each beetle was scheduled to be tested for each substance once (appendix 2). The second and third experiment involved fifteen beetles, seven identified as male, eight as being female. Fourteen beetles were tested each day. Once again, each beetle was tested only once per day and once per substance. In five days, each substance was tested on fourteen beetles, seven males and seven males.

At the start of each trial, the (mixture of) chemical(s) and the control were placed at the end of the two arms of the Y-tube. Beetles were subsequently released into the Y-tube at the bottom of the main arm. Beetles preferred walking upside down on the top of the tube. However, some beetles were too heavy and would fall down repeatedly or not get on top at all. Once at the fork, beetles could intercept volatiles coming from both arms. In the first experiment, beetles often simply seemed to keep walking on whichever side they entered the Y-tube. In the other two experiments, beetles would often vigorously wave with each of their antennae in a different arm before walking into one of them (figure 7).

4.1.1 Supplementary feeding and choice

At the start of the first experiment, June 12th, it was not clear which tree branches from the Beijing Forestry University campus would be eaten by the beetles. The beetles were supplied with, but did not eat, from *Salix babylonica*. The bark of *Acer truncatum* was eaten by the beetles.

4.1.2 Gender identification

Before the experiments, gender identification was performed on sight by examining the ventral side of the last antennomere (antennal segment), which is partly white in females, whereas is it usually fully black in males. The gender of the beetles used for the second and third experiment was double-checked by also examining whether the abdomen was exposed, as it is in females, or unexposed, as in males. Beetles were only included in those experiments, when both characteristics indicated the same sex.

After the experiments, the gender of the beetles was identified by dissection (figure 8). 79% of the beetles in the first experiment (11 out of 14 beetles) was correctly identified by sight. All erroneously identified beetles had been identified as males, but were determined to be female after dissection. 100% of the beetles in the second experiment (16 out of 16 beetles) was correctly identified.

Fig. 7 Beetles walking upside down on the top of the glass, while extending their antennae into the different arms. Photos by Eline Verbon.

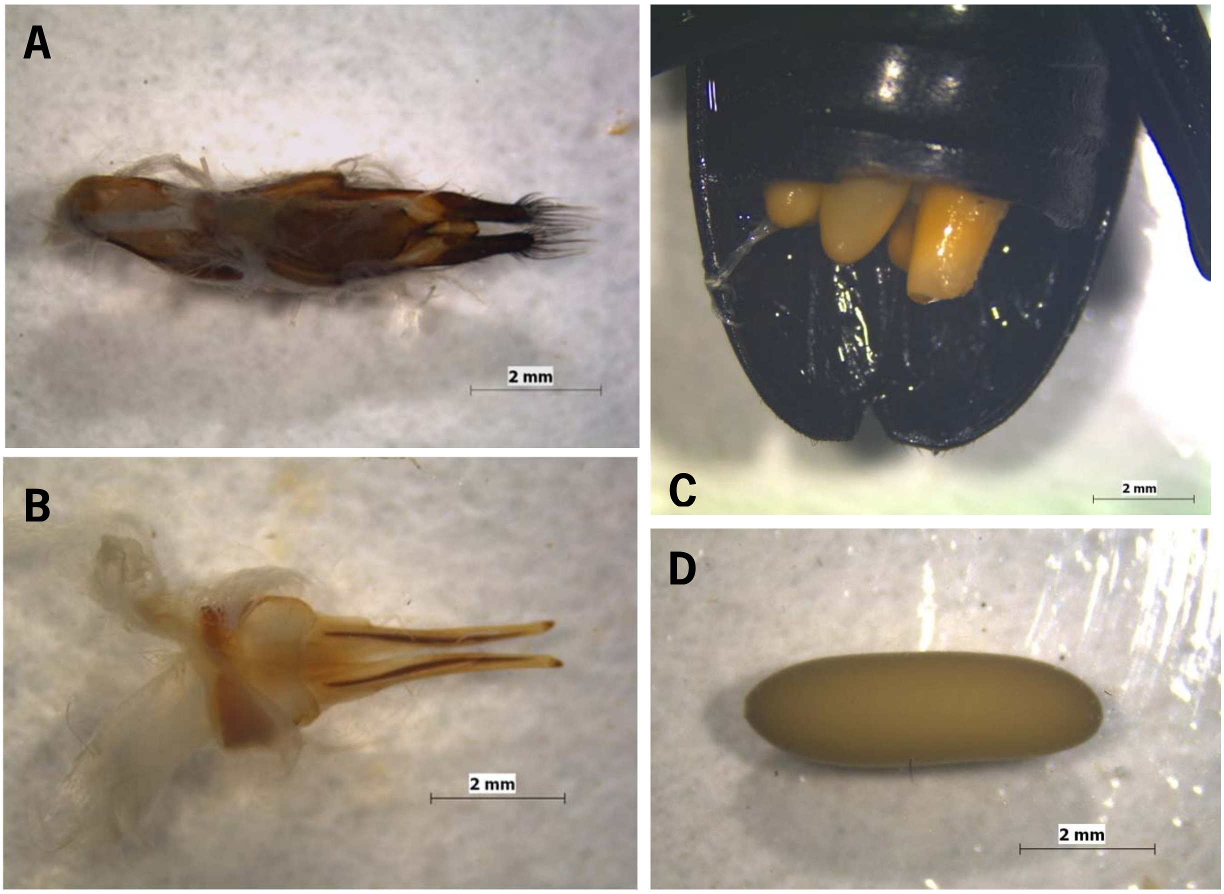


Fig. 8 The genital organs of A) male and B) female *A. chinensis*. C) A female abdomen, with the last two segments cut off, thereby exposing her eggs. D) An *A. chinensis* egg. Photos by Eline Verbon.

In the first experiment, average body length (measured from start of antennae to top of wings) of males was 2.8±0.25 (n=4) and females was 2.8±0.36 (n=10). All males died between June 14th and June 16th. Females died from June 14th up to July 2nd. The males participating in both the second and third experiment were on average 2.9±0.20 (n=7) and females 3.1±0.43 (n=8). The two beetles that had died before July 2nd were both female.

4.1.3 Attractiveness of β-caryophyllene, α-humulene, linalool and cis-3-hexen-1-ol in 10^{3} dilutions

The first experiment was not completed due to severe weakening of the beetles by the fourth and final day of the experiment, probably because they were underfed. Therefore, on the fourth and final day of this experiment, all trials had to be cancelled. In total, nine beetles were tested for each volatile. All these beetles showed a response before an hour had passed, with a maximum decision time of 39 minutes. In most trials (69%) beetles showed a response, i.e. passed the black connector at the end of either of the arms, within 10 minutes. The 11 trials in which beetles that took longer to decide were equally distributed among the test substances (twice A, trice B, twice C, four times D). Beetle number 11 and 12 took more than 10 minutes twice. Beetle number 4 was the only beetle taking more than 10 minutes for a choice three times. None of these beetles died shortly afterwards. Thus, the long decision time was not a sign that the beetles were unhealthy. In fact, beetle number four was the beetle that lived longest while in captivity: more than two weeks after the start of the experiment. The choice of any one beetle for the right or the left arm was not dependent on the choice of the previous beetle (figure 9).

Females were significantly more attracted to α-humulene ($\chi^2=5$, $p<0.05$) (figure 9). No repellent or attractive effect was elicited by any of the other chemicals on either male or female beetles.

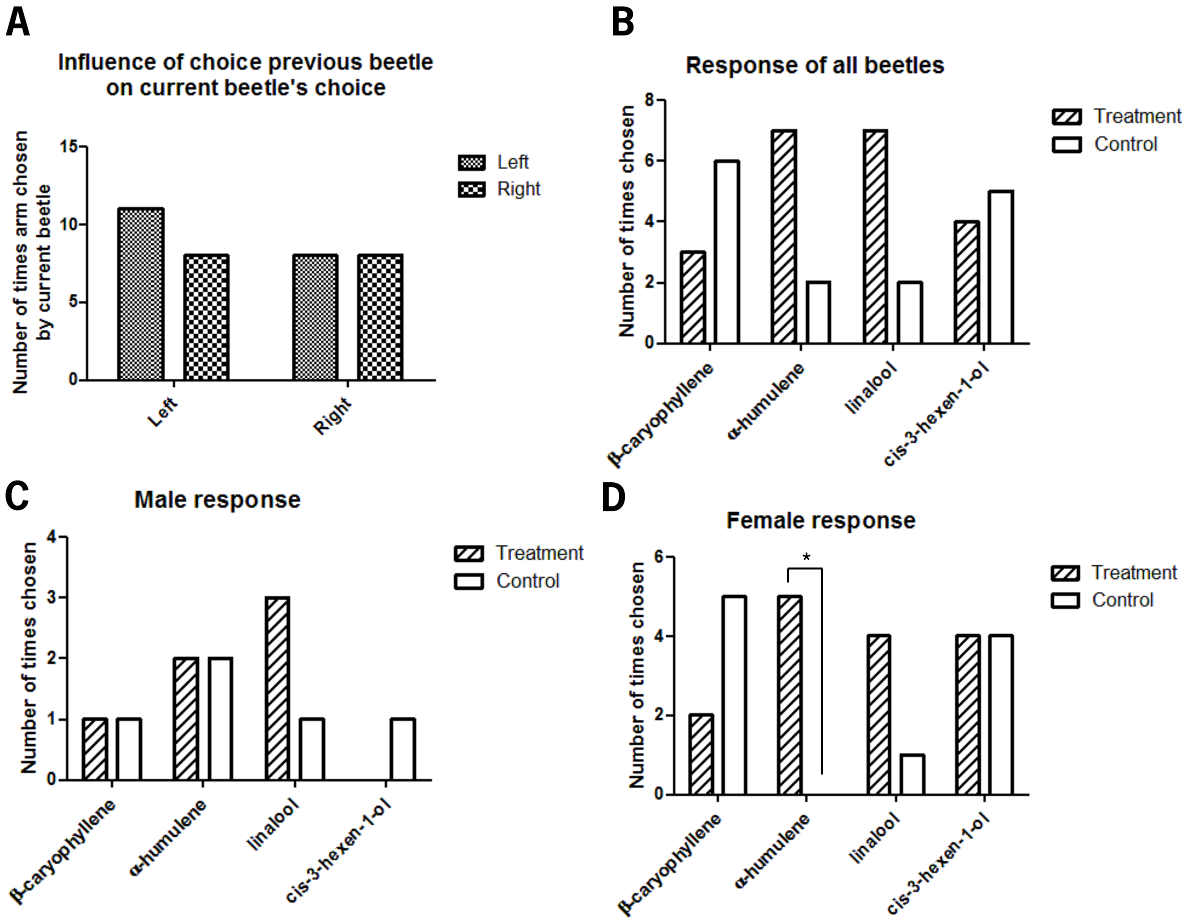


Fig. 9 First experiment: response of the beetles in trials testing single compounds in a dilution of 10^{-3} in paraffin liquid against the pure solvent. A) Whether the previous beetle chose the left or the right arm, did not influence the choice for either the left or the right arm of the current beetle (N=36) (χ^2=0.47, p>0.05; χ^2=0, p>0.05). B) There was no effect of any of the substances on all the beetles taken together (each chemical, N=9). None of the chemicals attracted or repelled males (β-caryophyllene: N=2, α-humulene, linalool: N=4, cis-3-hexen-1-ol: N=1), whereas D) α-humulene was attractive to females (N=5) (χ^2=5, p<0.05), while β-caryophyllene (N=7), linalool (N=5) and cis-3-hexen-1-ol (N=8) elicited no response.

4.1.4 Attractiveness of β-caryophyllene, α-humulene, linalool and cis-3-hexen-1-ol in 10-1 dilutions

In 97% of the trials (68 out of 70) a response was recorded, either test or control. In the other two cases, 1 hour passed before a beetle had passed the black connector. Both of these beetles did move around and both stayed at the fork for a while. In their other trials, they were not noticeably slower than the other beetles. Of the trials that resulted in a response, 68% of the trials (46 out of 68) were carried out within 10 minutes. The trials taking more than 10 minutes were not non-evenly distributed (χ2=5.35, p>0.05) (figure 10). The Y-tube was symmetrical and the choice of any one beetle to be independent of the choice of the previous beetle (figure 10).

None of the substances showed a clear attractant or repellent effect on either male or female beetles (figure 10).

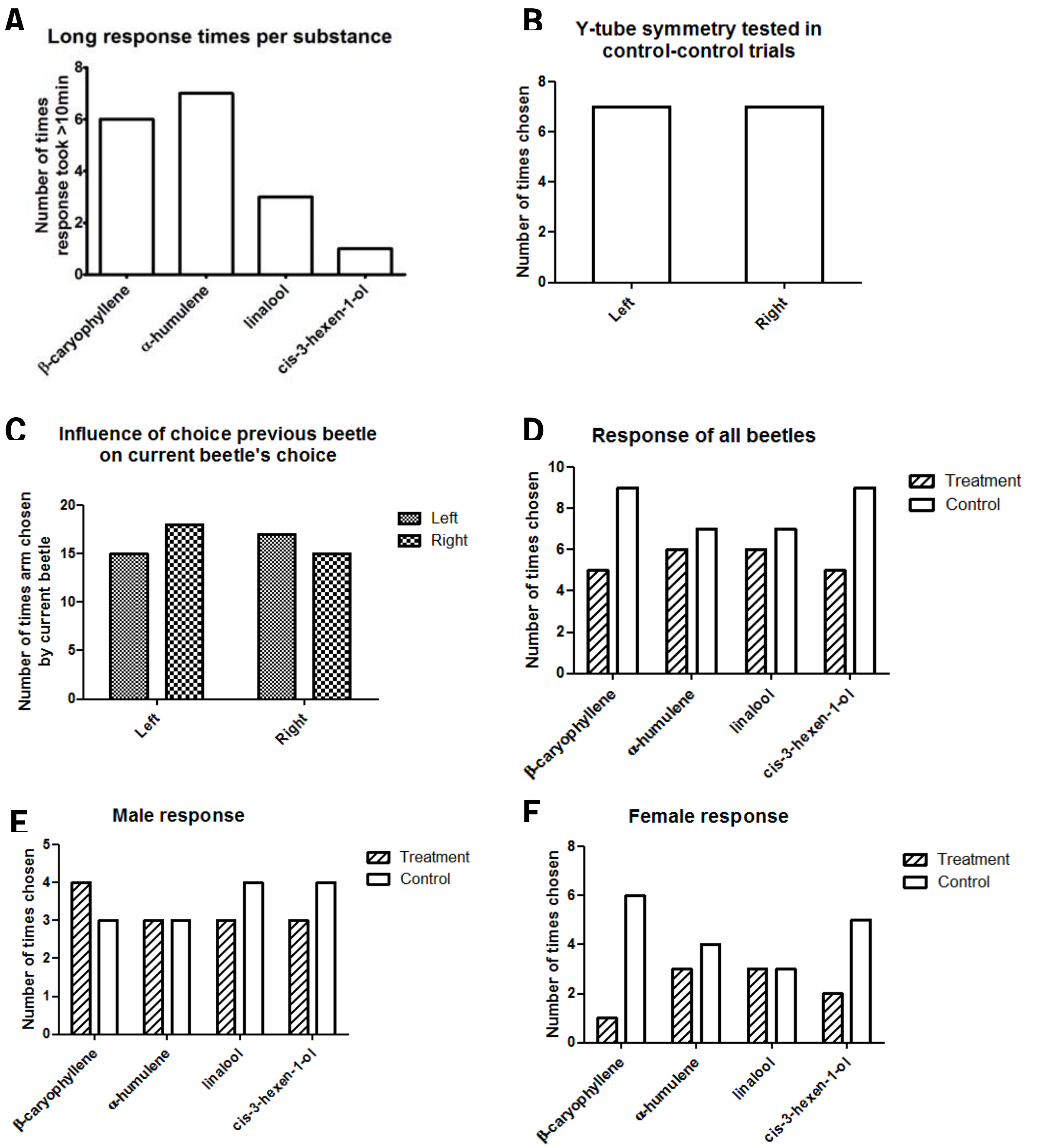


Fig. 10 Second experiment: response of seven male and seven female beetles in trials testing single compounds in a dilution of 10^{-1} in paraffin liquid against the pure solvent (N=14, n=5). A) The trials taking more than 10 minutes were not equally distributed among the different substances. B) The Y-tube was shown to be symmetric in control – control trails (N=14). C) The choice of the current beetle for the left or the right arm was not influenced by the choice of the previous beetle. D) There was no clear attractive or repellent effect for any substance when analysing all beetles, E) nor when analysing males' F) or females' responses separately.

4.1.5 Attractiveness of chemical mixtures

During the third, fourth and the fifth day of the third experiment temperatures in Beijing had decreased from over 30°C to 20-25°C. The activity of the beetles decreased noticeably during these days. On the fourth and fifth day, Y-tube experiments were no longer useful because beetles did not move from the insertion point. Thus, only eight replicates were tested per volatile. 10% of all the executed trials (4 out of 40 trials) resulted in no response: two beetles had been in the Y-tube for an hour, one beetle had remained motionless at its point of entry for half an hour and one beetle toppled over and could not get back on its feet. No significant effects were elicited by any of the mixtures (figure 11).

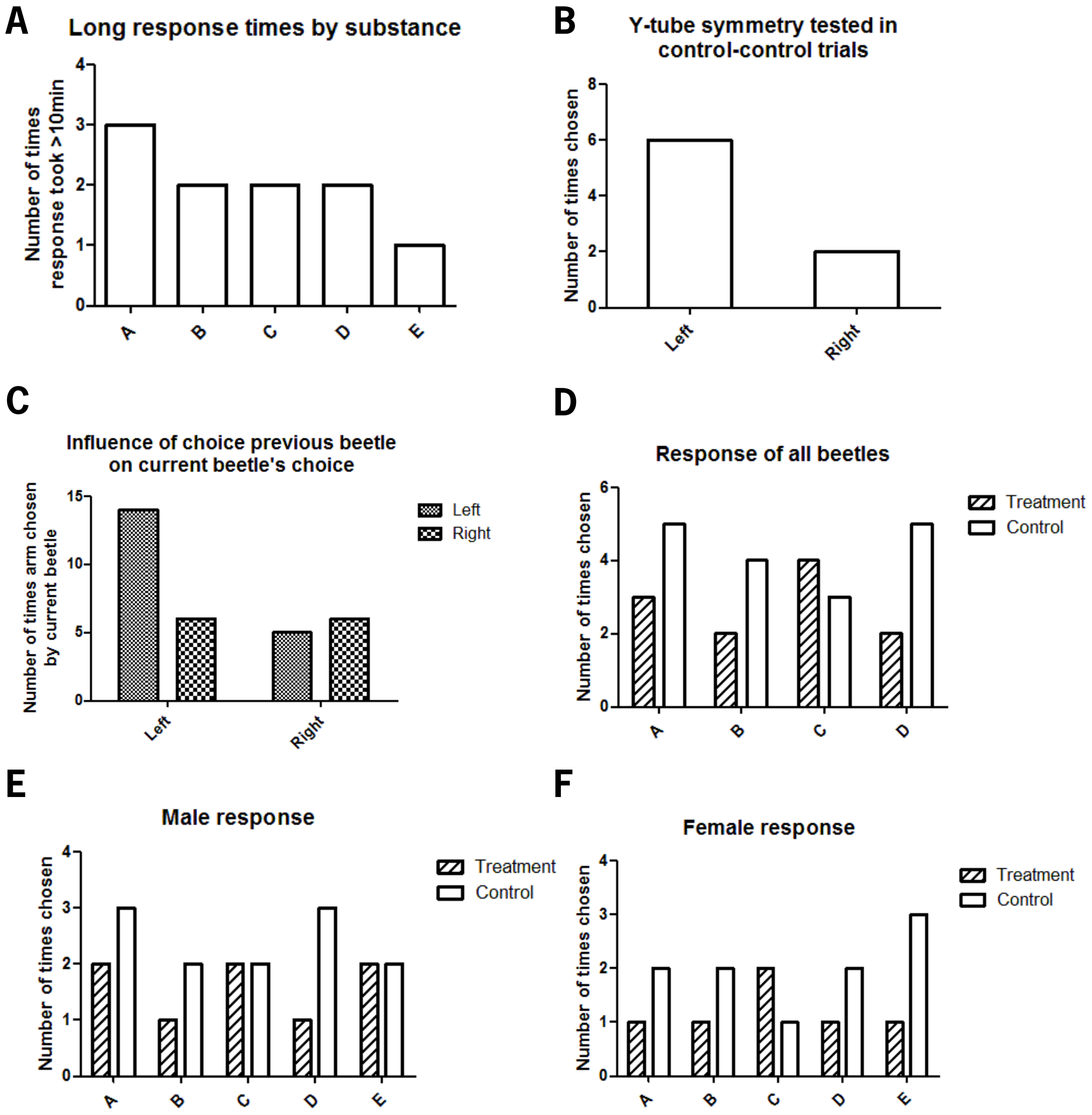


Fig. 11 Third experiment: response of seven male and seven female beetles in trials testing mixtures of the single compounds in a dilution of 10^{-3} in paraffin liquid against the pure solvent (N=40). A) The trials taking more than 10 minutes were equally distributed among the different substances. B) In eight control – control trials, beetle chose the left arm more often, but this effect was not significant (χ^2=2, p>0.1). C) The left arm was chosen more frequently only when the previous beetle went left as well. This was also not significant (χ^2=3.2, P<0.1). D) There was no clear attractive or repellent effect for any substance when analysing all beetles, E) nor when analysing males' and F) females' responses individually. *A: β-caryophyllene + α-humulene; B: linalool + cis-3-hexen-1-ol; C: : β-caryophyllene, α-humulene + linalool; D: β-caryophyllen, α-humulene, linalool + cis-3-hexen-1-ol.*

4.1.6 Evaporation of the chemicals

Evaporation of the chemicals from the septa, measured as their weight loss, was not uniform throughout the experiment. Evaporation was high just after leaving the freezer and got progressively less during the hours afterwards. From approximately 2 – 4 hours after taking the chemicals from the freezer, the evaporation rate seems relatively high and constant (figure 12). Similar data were measured for chemicals made from either frozen pure chemicals, subsequently diluted in paraffin liquid, or from frozen dilutions, neither of which were frozen again after loading onto the septa (appendix 5 and 6). These latter two graphs show a cumulative weight loss that is higher than 100mg, the amount of chemicals that was added to the septa. Possibly, the hexane, in which the septa were cleaned, did not completely evaporate before the septa were loaded. This did not influence the trend of evaporation.

4.2 Field experiment

On July 5th, 1 beetle was caught in one of the control traps. Another beetle was caught in a trap baited

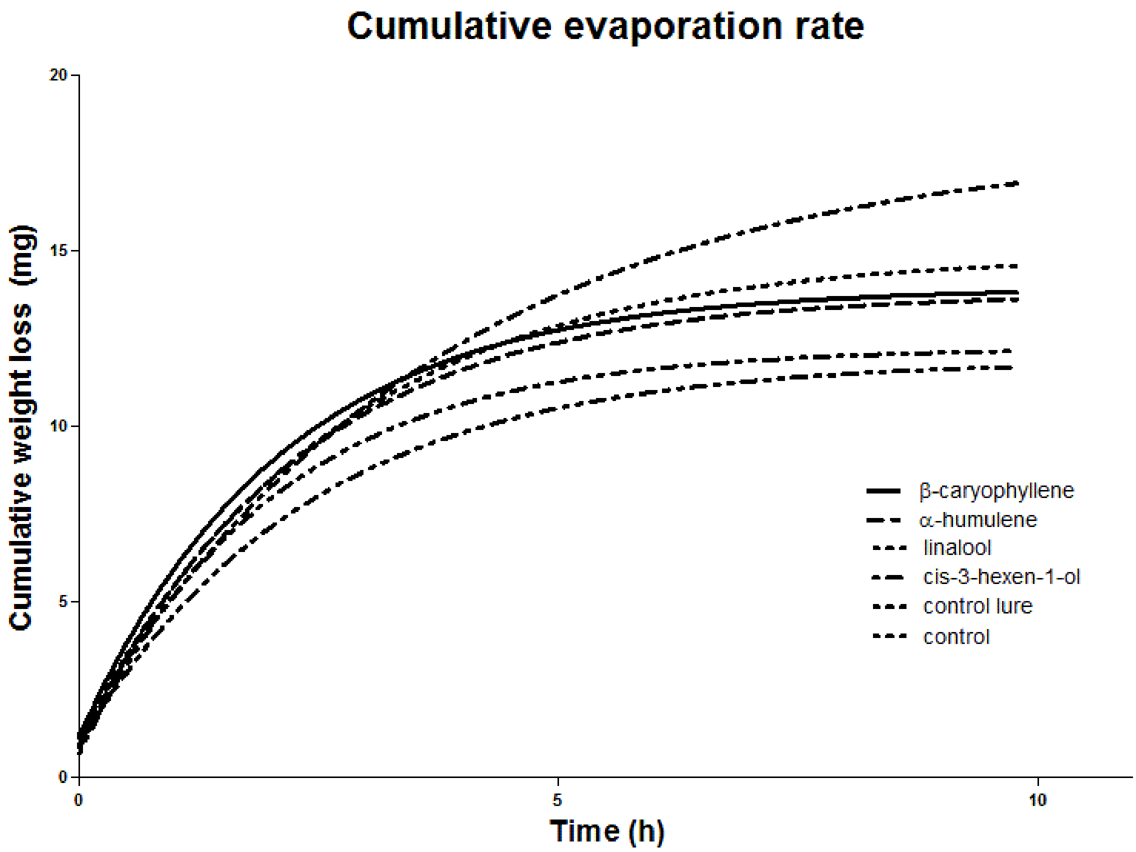


Fig. 12 Cumulative evaporation rate of the 10^{-3} single compound dilutions in the rubber septa during the second day of the second experiment. Evaporation rate is measured as weight loss.

with the mixture of linalool and cis-3-hexen-1-ol. On July 21th more than half of the traps had either fallen down or fallen apart. The bottoms of the remaining traps were all filled with water. No beetles were collected in any of the traps on this day. On July 28th, all traps were in good condition. 1 beetle was found in a control trap.

There were no significant by-catches. We saw beetles in the surroundings, but did not count or note the numbers.

5. Discussion

Anoplophora chinensis (Forster) is part of the family Cerambycidae (Coleoptera) and has recently been synonymised with *Anoplophora malasiaca* (Thomson) (Lingafelter & Hoebeke, 2002). *A. chinensis* is native to South and East Asia, where it is considered to be a pest species: invasion of *A. chinensis* into a forest or tree plantation can cause massive tree death (Wang, 1998; Haack et al., 2010, Hanks, 1999). Invasion of *A. chinensis* in nonnative territories is therefore a major concern. Even more so because *A. chinensis* has been intercepted several times in Europe and the USA the past few years. Beetles were imported into these countries within living wood packaging material imported from Asia, mainly China (Haack et al., 2010). To prevent establishment of *A. chinensis* within Europe and the USA rapid and efficient detection of the beetle is necessary. Currently, the methods of detection still rely on visual inspection of trees. Invading beetles are often detected accidentally by laymen (Hérard et al., 2006).

A widely investigated method of Cerambycidae detection is by attracting the beetles to traps with lures composed of natural volatiles. These lures are often either host plant volatiles, sex pheromones or a combination of both (for examples, see Nakamuta et al., 1997; Nehme et al., 2009; Nehme et al., 2010; Reddy et al., 2005; Wickham et al., 2012; Yasui et al., 2007a). This project investigated the attractiveness of β-caryophyllene, α-humulene, linalool and cis-3-hexen-1-ol as possible attractants for *A. chinensis*. β-caryophyllene and α-humulene function as both long-distance and short-distance attractants for male *A. chinensis* (as *malasiaca*) beetles. The volatile is probably emitted by *Citrus unshiu*, a host plant of *A. malasiaca*, and taken up by the elytra of the females via the air (Yasui et al., 2007a). (-)-linalool and (Z)-3-hexen-1-ol are plant volatiles that function as long distance attractants for *Anoplophora glabripennis* (Motschulsky), the closest relative of *A. chinensis.* These volatiles are present in air emitted by a large range of host trees in the USA, and absent in air emitted by non-host species. The volatiles were shown to attract beetles in the field (Li et al., 2003; Wickham & Teale, as cited in Nehme at al., 2009; Nehme et al., 2010).

The four compounds were tested both in the Y-tube olfactometer and in the field. A Y-tube olfactometer measures mostly short-distance attraction, whereas a field experiment can prove long-distance attraction. Since *A. malasiaca* and *A. chinensis* are considered as one species, we expected β-caryophyllene and α-humulene to be attractive to *A. chinensis* mainland males in the Y-tube olfactometer and the field as well. Since *A. glabripennis* is the closest relative of *A. chinensis*, linalool and (Z)-3-hexen-1-ol were also expected to be attractive in the field experiment to both sexes.

In the Y-tube olfactometer, beetles were released at the far end of the main arm in all 3 tests. When walking upwind, against the airflow, they had the choice between an arm containing a possible lure and an arm containing a control, i.e. the pure solvent paraffin. The tested chemicals consisted of single volatiles in a 10^{-3} or a 10^{-1} dilution in paraffin liquid, or mixtures of different volatiles, again in a 10^{-3} dilution in paraffin liquid. Mixtures tested were 1) β-caryophyllene and α-humulene, 2) linalool and cis-3-hexen-1-ol, 3) β-caryophyllene, α-humulene and linalool and 4) all four volatiles: β-caryophyllene, α-humulene, linalool and cis-3-hexen-1-ol.

In the first two experiments, the Y-tube was shown to be symmetrical and the choice of the previous beetle, left or right arm, was shown not to influence the choice of the current beetle. Unfortunately, the first experiment, with the 10^{-3} dilutions, could not be carried out fully because the beetles involved had become too weak by the fourth, and last, day of the experiment. The beetles had become weak because they did not eat enough. Too few *Melia azedarach* L. branches were brought from Fujian to feed the beetles during the test period and the beetles did not eat from the *Salix babylonica* L. branches collected in Beijing. They did feed on *Acer truncatum* Bunge, which is consistent with previous literature on *A. chinensis* tree preference when it invades other territories (Peverieri & Roversi, 2010). Thus, although *A. chinensis* has a broad host range, not all branches are suitable for feeding in laboratory conditions.

During the first experiment, only nine replicates were tested per volatile, instead of the twelve that was aimed for. Moreover, the male:female ratio was not 50:50 for each volatile, due to incorrect gender identification by exterior characteristics. Additionally, the beetles had gotten weaker throughout the experiment and had been subjected to different foodstuffs. Differences in food intake will affect the response of *A. chinensis (malasiaca)* to attractant chemicals (Yasui et al., 2011). Our results indicate that α-humulene and linalool might be attractive to the beetles (both N=9; χ^2=2.78; P<0.1). Female beetles are attracted to α-humulene (N=5; χ^2=5, p<0.05). This is surprising, since the conspecific *A. malasiaca* is attracted to both β-caryophyllene and α-humulene (Yasui et al., 2007a) and *A. chinensis'* closest relative *A.*

glabripennis is attracted to linalool and cis-3-hexen-1-ol (Nehme et al., 2009). Therefore, I expected all four chemical to function as attractants for *A. chinensis*. Due to the small number of replicates and the abovementioned problems, no final conclusions can be drawn from the results of the first experiment. Further research with a larger number of replicates and homogenous food and temperature conditions is necessary.

During the first experiment, many beetles simply seemed to rush through the Y-tube within one minute without taking time to sense different volatiles. That might indicate that the concentration was too low to be sensed by the beetles. Therefore, during the second experiment, the concentration was raised a 100-fold to 10^{-1}. Using this concentration, none of the substances showed an attractant or repellent effect neither on male beetles nor on all beetles. β-caryophyllene might even be repellent to females in this concentration (N=7; χ^2=3.57, p<0.1).

The third and last experiment was not completed because the activity of the beetles dropped to such an extent that further experiments were not possible the last few days. These days were characterized by heavy rain during the first night, relatively low temperatures, approximately 25°C, and cloudy skies. The lower temperature may have been the decisive factor; temperature is known to influence adult survival and reproduction of *A. glabripennis* in the USA (Keena, 2006). This is in accordance with the finding that beetles that invade cooler regions, such as the Netherlands, do not spread further than 40 m in their life (A. Loomans, personal correspondence). No attractive or repellent effects were observed. This might be due to the low number of replicates.

Although possible, it seems unlikely that none of the chosen chemicals had any effect of the beetles. Instead, seemingly random choice of beetles between test compounds and controls might be caused by the storage of all septa within the same box while in the freezer. This might have caused mixing of the volatiles. Moreover, freezing the chemicals until just before use might have affected evaporation rate. We did measure evaporation rate as the amount of weight loss over time. However, since we did not have the facilities to measure the amount of volatile in the air in the Y-tube, no conclusive evidence can be presented on the release rate of the volatile in the tube. This might have been too low for the beetle to perceive. Moreover, many factors could have interfered with the beetles' choice. For example, the food that beetles feed on has a large influence on attractiveness of chemicals (Yasui et al., 2011), as does mating status (Wickham & Teale, 2009) and probably many other factors that have not yet been identified. Finally, the number of replicates in this study was probably too small to achieve statistically significant results.

In the field, natural odours were tested as lures within cross-vane panel traps. The following mixtures were tested in seven replicates: 1) α-humulene and linalool, 2) α-humulene, linalool and β-caryophyllene, 3) α-humulene, linalool and cis-3-hexen-1-ol , 4) humulene, linalool, β-caryophyllene and cis-3-hexen-1-ol and 5) control. Each chemical mixture was composed of pure chemicals with a total volume of 200µl per trap in the first two weeks and 400 µl in the last week. None of the chemical-trap combinations resulted in significant trap catches. Likewise, the number of bycatches was insignificant. This might indicate that the chosen mixtures do not attract *A. chinensis* at long distances, at least not in the amounts presented. In addition, the trap used, i.e. the cross-vane panel trap, might not be suitable to catch *A. chinensis*, or beetles might be attracted to the surroundings of the trap, without being caught. A study by Yasui et al. (2007a) showed an increase of the number of beetles at .7 to 1.2 m distance from their lures, although no beetles were caught in their traps. In this project beetles might have been attracted to the trees or direct surroundings without actually entering the trap.

This project has first of all provided basic knowledge on research with *A. chinensis*. For example, it has shown that keeping the beetles at the right temperature, around 30-35°C, is extremely important. Moreover, beetles were shown to be specific about the food they eat. The beetles used in this project would not eat *Salix babylonica*, although this species is used as a host in Europe, but did eat *Acer truncatum*. Of the latter, the bark of the branches was the preferred food source. Preferably, this should be validated for beetles of further experiments before starting experiments, since different food stuffs can result in different preference for volatiles. This project has given an indication that linalool and α-humulene are attractive to female *A. chinensis* in Y-tube olfactometer essays. Field experiments did not give any significant results for the lures used.

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Bijlage 4 Poster gepresenteerd op 2nd International Congress on Biological Invasions

WAGENINGEN UNIVERSITEIT
WAGENINGEN UR

Application of several technologies in the production chain in China to control the *Anoplophora* longhorn beetles

Yu Tong Qiu§, Jos van Meggelen and Antoon Loomans

Background: In recent years citrus longhorn beetle (CLB) *Anoplophora chinensis* and Asian longhorn beetle (ALB) *A. glabripennis* were accidentally introduced with imported woody plant and packing wood materials from China into the Netherlands[1]. The Dutch government was forced to take quarantine measures which caused sever economic loss. In order to reduce the risk of CLB and ALB infection in the host plant products packing wood materials in China, the Dutch ministry of Economy Agriculture and Innovation has initiated a research project to tackle the CLB and ALB problem jointly with the Chinese authority AQSIQ.

Project activities: In this joint research project monitoring and control technologies will be tested to evaluate the feasibility of application in China. For monitoring and control purpose lure-baited traps will be tested for the effectiveness in catching adult CLBs and ALBs[2,3]. In an earlier study, X-ray scan has been shown to be able to detect beetle larvae inside stem or branches of woody plants[4]. This technology will be evaluated for the detection of CLB larvae in host plants or ALB larvae in wood materials. Earlier research had proved a controlled atmosphere temperature treatment (CATT) to be effective in killing mites and nematodes in strawberry plants[5]. The CATT technology will also be tested for the applicability in killing various stages of CLBs and ALBs in woody plant materials.

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Netherlands Food and Consumer Product Safety Authority, P.O. Box 9102, 6700 HC, Wageningen, The Netherlands.
Email: j.c.van.meggelen@minlnv.nl; Tel. +31 0620013622
Email: a.j.m.loomans@minlnv.nl; Tel. +31 0317 496825

Bijlage 5 Letter of Intent between WUR and CAIQ

Between
Wageningen UR, whose business address is at Droevendaalsesteeg 1 (6708 PB) Wageningen, The Netherlands

And
Chinese Academy of Inspection and Quarantine (CAIQ), established in Beijing, China, whose business address is at No. A3, Gaobeidian North Road, Chaoyang District, Beijing, China.

Wheras Wageningen UR (WUR) is a cooperation between Wageningen University and the foundation Stichting Dienst Landbouwkundig Onderzoek.

1. Purpose

1.1. This Letter of Intent (LoI) establishes an arrangement between the foundation Stichting Dienst Landbouwkundig Onderzoek, research institute PPO/PRI, and the Chinese Academy of Inspection and Quarantine (CAIQ), hereafter referred as parties.

1.2. This arrangement establishes a collaborative relationship between the Parties in connection to the future development and advancement of the research outlined under section 2 of the LoI.

2. Research

2.1. The Parties intend to collaborate in, but will not strict to, the following:

(a) Explore future possibilities for exchange in research staff members and students between the Parties

(b) Jointly organise seminars, symposia and bilateral talks on subjects related to phytosanitory issues. Joint research projects on subjects of common interest, including joint application for national and international projects

(c) Further co-operative research projects as determined by the Parties.

3. Duration

This LoI will take effect on the day of execution and will terminate on 31 December 2017. The Parties will use reasonable endeavours to extend the agreement beyond 31 December 2017.

4. Administration

4.1. The co-operative arrangements in the LoI will occur without costs or charges being levied between the Parties. Any costs or charges to be levied against either Party will be discussed on a case by case scenario before any costs are incurred by either Party.

4.2. A variation to the LoI may be proposed by a Party, but must be in writing and mutually agreed upon by the Parties prior to the variation coming into effect.

4.3. The LoI will come into effect on the day on which it is signed by both Party.

4.4. A Party may terminate the LoI by written notice to the other Party. The LoI would terminate 30 calendar days after the date upon which the Party receives written notice of the intention to terminate.

If this LoI results in a project relating to phytosanitory issues, the following conditions shall apply, unless otherwise agreed to by parties in a cooperation agreement.

5. Access to and exchange of Information

5.1. Subject to sub-section 5.2, the Parties will make available, upon request in writing, any confidential information or proposals for the purpose of collaborative research both nationally and internationally.

5.2. Information provided by either party under sub-section 5.1 will be provided on a confidential basis. This information will not be released to any person other than members of either Parties who requests the information for the purpose of collaborative research, except after receiving written consent from the Party providing the confidential information.

6. Publications and Intellectual Property

6.1. All inventions, discoveries and other knowledge and/or information made solely by either Party shall be the property of the generating Party, and those made jointly shall be joint properties.

6.2. Any patents or patent rights resulted from or in connection with the collaborative research that are jointly invented by the Parties shall be jointly owned by said Parties.

6.3. Subject to the obligations of and restrictions upon each of the Parties, each Party is free to carry out any related research and/or to co-operate with any third party as long as such research and/or co-operation shall not jeopardize or harm any other Party's interest under this Agreement.

7. Contact Persons

7.1 WUR: Dr. Yu Tong Qiu

7.2 CAIQ: Prof. Yuejin Wang

Bijlage 6 CATT proef met Japanse thujabastkever

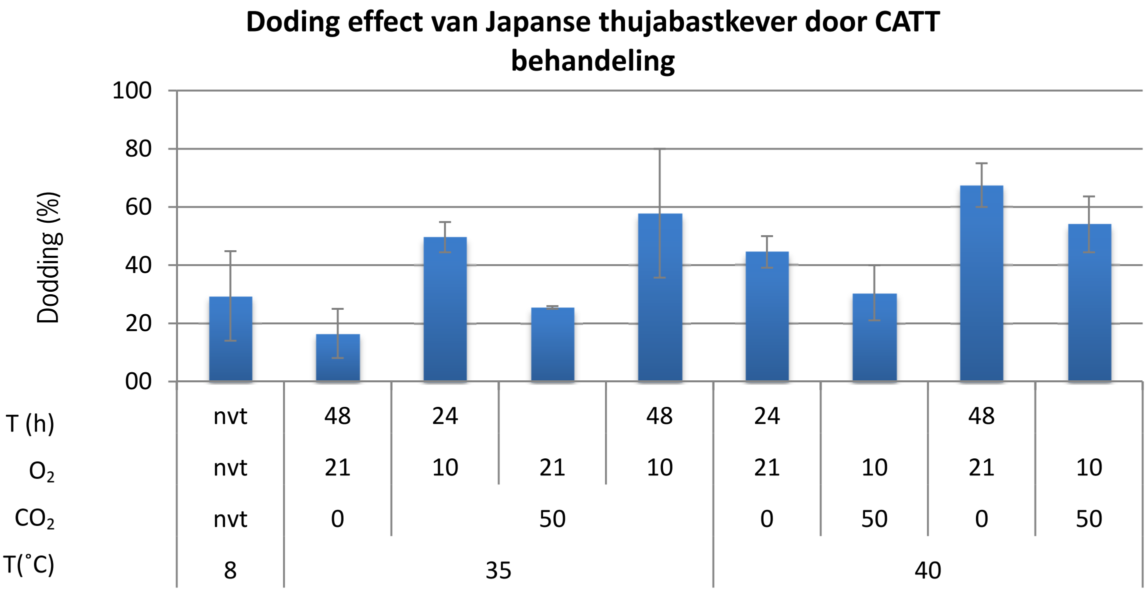

Figure 1 Thuja stam besmet met Japanse thujabastkever, *Phloeosinus rudis* wordt onder 8 CATT condities behandeld. De doding effect van de CATT behandeling is van dit proef niet duidelijk aangetoond.

Bijlage 7 Business plan voor samenwerking met CAIQ

Introduction

CAIQ and Wageningen UR (PSG) have recently signed a Letter of Intent. In order to fill in the content of the collaboration between the two parties, we have made a proposal for two projects. Each project deals with the application of a specific technology on the control of quarantine pests that are important for both China the The Netherlands, such as the Citrus Longhorn beetle.
Both projects will be carried out mainly by researchers from CAIQ and WUR. AQSIQ and NVWA will participate as advisors. Technical companies, such as Philips and Fruitong, will participate as a third party who may provide technical support (knowledge and technical facilities, such as equipments).
Both projects aims at the development of a practical solution of quaratine pest problems.

Project I. X-ray detection of Citrus Longhorn Beetle and other quarantine pests of woody plants
Product

- Scanning device for detecting boring insects, such as Citrus Longhorn Beetle in small living trees (Length (max 1 meter), bunch and roots with diameter of max 50 cm).
- 3D x-ray or CT with simplified approach and automated image analysis.
- Fully automated detection of single trees.

Output: OK, Not OK (holes detected), suspicious (further manual investigation needed)
Target price < € 50.000

Application target

- Scanning before planting at grower production site before entering net-house (China)
- Export scanning at grower production site (China)
- Export scanning by local Chinese authorities (China)
- Import scanning by European/Dutch authorities (Netherlands)
- Import scanning by European/Dutch growers (Netherlands)

Project
Phase 1. Feasibility study

1. Realise measurement setup with Philips equipment
2. Test set of 500 plants with at least 100 plants infected
3. Image acquisition
4. Development of algorithms
5. Classification and score
6. Design and plan implementation
7. Evaluation and Go / No Go

Phase 2. Prototype development

1. Design and realisation of prototype
2. Practical trials
3. Improvement steps
4. Final prototype

Phase 3. 0-serie and implementation

1. Final design production machine
2. Service and instructions
3. Roll out of product

Project II. CATT application as phytosaitary treatment quarantine insect and nematode pests (such as Citrus Longhorn beetle)
Product:

- CATT R&D and demonstration facility.
- CATT protocols of a phytosanitary treatment as a quarantine control measure.
- Implementation CATT method in practice.

Application target

- Quarantine treatment export plants with potential insect and nematode problems (China)
- Quarantine treatment import plants with potential insect and nematode problems (Netherlands)

Project
Phase 1. CATT research implementation

1. Realise CATT research setup with CAIQ and Fruitong
2. Dry test setup, technical optimisation
3. Initial test CATT against insects/nematodes
4. Fytotoxicity tests of CATT with target plants
5. Evaluation and Go/No go

Phase 2. CATT target pest treatment development

1. Practical trials series 1 (pests & plants)
2. Practical trails series 2 (fine-tuning)
3. Improvement series 3 (initial scale-up, pests and plants, cultivars etc)
4. Final CATT pests with plants

Phase 3. CATT scale-up and implementation

1. Design CATT facility
2. Building and testing commercial CATT facility
3. Practical trial CATT implementation

Bijlage 8 Programma voor bezoek aan Thailand

Program - Thailand BOCI 2013
Thrips Management in Orchids Exported from Thailand to EU
Thursday 28th November – Mon 2nd December 2013

(Mon 25th Nov 2013 / 13.30 hr. last modified)

Delegations

1. Dr. Cornelis (Kees) Booij
   Plant Research International – Wageningen UR, The Netherlands
   Email: kees.booij@wur.nl
2. Ms. Qiu Yutong
   Plant Research International – Wageningen UR, The Netherlands
   Email: yutong.qiu@wur.nl
3. Mr. Sarawut Ch. (coordinator)
   Agricultural Section - Netherlands Embassy, Bangkok
   Email: ks.chantachitpreecha@minbuza.nl
   MB 66.89.2057131

Thu 28 Nov

06.45 Arrive Bangkok
Transfer to Hotel (?) by public taxi
Note: Early check in at the hotel is required

12.00 Pick up at the hotel by the Embassy car

13.00 Meeting with Thai DOAE
Venue: Department of Agricultural Extension (DOAE)
Ministry of Agriculture and Cooperatives
2143/1, Paholyothin Rd., Chatuchak, Bangkok
Coordinator: 1. Ms. Chanya Dibanuka
Chief of National Orchid Board Secretariat
Email: cdibanuka@hotmali.com
MB 66.86.6279463
2. Ms. Sunisa Boonyaoatipark
Chief Foreign Relations Section – DOAE
Email: sueboonya@hotmail.com
MB 66.89.2065260

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16.00	Transfer to the Netherlands Embassy by the Embassy car Venue: 15, Soi Ton Son, Ploenchit Rd., Bangkok
17.00	Courtesy meeting with the Netherlands Ambassador (H.E. Mr. Joan Boer) Venue: The Netherlands Embassy

Fri 29 Nov

08.15 Pick up at the hotel

Visit 2 orchid growers in Nakorn Pathom Province

Coordinators: Ms. Sasitorn Punsiri (Managing-Director)
S.P.F. International Co., Ltd.
300/9, Soi Lard Phrao 1, Lard Yao, Chatuchak, Bangkok 10900
Email: sasitorn_p@hotmail.com
MB 66.81.8664283

Note:

- S.P.F. International Co., Ltd. is the importer and exporter in flowers & bulb. The two orchid growers are the suppliers of S.P.F. International Co., Ltd.
- Ms. Oratai Euatrakool (Thai DOA) will be joining the farm visit.

15.00 Depart to Suvarnabhumi International Airport, Bangkok

17.00 – 19.00 Visit the export inspection at Suvarnabhumi International Airport Plant Quarantine Station, Department of Agriculture (DOA)

Coordinator: Ms. Oratai Euatrakool (Senior Agriculturist)
Export Inspection Services – Office of Agricultural Regulation, DOA
Email: oratai_doa@yahoo.com
MB 66.81.9337049

Note: Ms. Oratai suggests to visit the Plant Quarantine Station at the Airport in the evening because more activities and business are operating for the export.

19.00 Depart to the Hotel

Sat 30 Nov

08.00 Depart from Suvarnabhumi International Airport (TG 102)

09.20 Arrive Chiang Mai International Airport

09.30 Visit Siam Orchid Culture Co., Ltd. and meeting with Mr. Nikon Kamyai (Owner & Managing-Director)

Venue: 135/3, Moo 12, Tambon Choeng Doi, Amphur Doi Saket,

Chiang Mai 50220
Email: nikorn@socthai.com ; nikorn@siamnobile.com
MB 66.81.9931316
www.socthai.com

Note: will visit Doi Inthanon (the highest mountain of Thailand) where natural low temperature treatment to the orchids has been introduced

18.00 Network Dinner with Orchid and Ornamental Horticultural Center - Maejo University and its orchid network.

Note:

- Venue to be decided later
- Coordinator Dr. Pranom Yangkhamman
  Deputy Director – Orchid and Ornamental Horticulture Center
  Maejo University, Amphur Sansai, Chiang Mai 50290
  Email yangkhamman@yahoo.com
  MB 66.82.9044177
  www.orchid.mju.ac.th

20.50 Depart from Chiang Mai International Airport (TG 121)

22.10 Arrive Suvarnbhumi International Airport
Transfer to the hotel by Airport Rail Link or public taxi

Sun 1 Dec FREE

Mon 2 Dec

07.30 Pick up at the hotel by the Embassy car
Depart Bangkok to Ratchaburi Province

09.30 Meeting with Mr. Jade Meeyanyieam
- President – Thai Orchid Exporters Association
- Owner & Managing-Director of Thai Orchids Co., Ltd.

Venue: Thai Orchid Co., Ltd. (Farm)
Amphur Ban Phao, Ratchaburi
Tel. 66.86.3408541 ; 66.85.0880103)

Note: Thai Orchids Co., Ltd. (Head Office)
41/1, Soi Bhudhabhucha 39 Yeak 1-1, Bang mod, Thung Kru, Bangkok 10140
Email contact@thaiorchidsgroup.com
www.thaiorchids.co.th

12.00	Depart Thai Orchids Co., Ltd.	
14.00	Meeting with Thai Department of Agriculture (DOA)	
	Venue:	Department of Agriculture Ministry of Agriculture and Cooperatives In Kasetsart University, Chatuchak, Bangkok 10900
	Coordinator:	Dr. Manita Kongchuensin (Senior Expert on Plant Pest) Email: manitathai@gmail.com MB 66.81.4250889
17.00	Depart DOA to the Hotel by the Embassy car	

Tue 3 Dec

02.05 Depart Suvarnabhumi International Airport to The Netherlands
Note: Hotel late checkout is required

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Contact: Sarawut Ch.
Agricultural Section
Netherlands Embassy – BANGKOK
ks.chantachitpreecha@minbuza.nl

(Mon 25th Nov 2013 / 13.30 hr. last modified)

Bijlage 9. Verslag bezoek aan Thailand betreft trips in orchidee productie

A picture of the orchid company structure in Thailand based on farm visits in Dec 2012 and Nov/Dec 2013.

Farms visited.
12 Dec 2012
The farm is a medium scale farm specialized in *Dendrobium* and some other orchid species. Tissue culture is the main source for new plantings and coconut shell is the regular growing medium. The farm has the complete production chain including a packing hall, an advanced fumigation facility and transport to the airport. Insect pest control is based on a weekly alternated chemical program. The chemical program and fumigation are considered as unavoidable to reach export quality. Any effective alternatives however would be welcomed. Workers in the plantations and packagers are trained to recognize systems and pests species that guide control and sorting of stems.

29 Nov 2013 Two orchid growers that are supplier to S.P.F International Co., ltd in Nakorn Pathom province
Farm A is a regular medium scale Dendrobium farm that gets breeding material from tissue culture supplied by the Tissue Culture Centre and produces a few varieties on a large scale for national as well as international markets. Grown flowers are transported to a central packing house where they are sorted on quality and fumigated to get rid of occasional Thrips infections. In the farm regular alternated insecticide sprays against insects (including thrips) are applied at a weekly base. No trapping system is in place but insects are scouted on a daily base by experienced managers when working in the plantations. Classical coconut shells are used as substrate and new system with plastic mats are tested. Quality and plant growth is managed by nutrient applications.
Farm B is a rather exceptional farm that is focused on breeding special varieties of many different species based on a long practice and fame of breeding and crossing.
To reduce chemical inputs for diseases experiments are done with undergrowth to change growing conditions of plants. More humidity at the base of the plant is expected to stimulate field resistance. Also Trichoderma is applied in the crop for disease control. Still disease and other pest problems remain. Insecticides are applied at a weekly base. The grower has the intention to change her company to a demonstration site for alternative and more biodiverse/green production system.
The farm has its own tissue culture production and MeBr fumigation chamber.
30 november 2013. SIAM Orchid Culture, Chiang Mai, mr. Nikorn Kamyai.
An orchid farm from an advanced and well-known variety breeder working for the national market who is transforming his farm to a large-scale farm that provides starting material and potted orchids to different parts of the world. New concepts of propagation and marketing of the hundreds of varieties available is the main strategy. Manipulation of growth/ blooming by low-temperature treatmenst are tested in the mountain elevations. Also propagation of plants by regrowth from cuttings is used in an upscaled system. Chemical treatments (twice a month) are applied to control various insects.
2 dec 2013 Thai Orchid co. Ltd, farm of mr. Jade Meeyanyieam
A modern farm with GAP certification that produces about 60 different species and variaties on a very large scale. The farm has an extensive own breeding program for introducing new varieties with new appearance (color, shape, fragrant) every year exporting cut orchid flowers to many countries such as Japan, USA and European countries. Yellow sticky traps are distributed at a large scale all over the farm. Scouting of insects is done by the farmers and insecticides applied focused on more infested plantings but on average on a weekly base. Emphasis is based on plant quality by manipulating nutriens and moisture. Keeping or planting trees and creating tree-zones at the farm to improve the mesoclimate and orchids grow well. The average temperature with tree zones can be up to six-Celsius degree lower than without. Sustainability is a target by having a green, clean and efficient farm, where it is good to work and a portfolio of high-end

flowers are produced. All export flowers are inspected and sorted on quality before sending them to a package house where final checks and fumigations takes place. The farm has a Tissue Culture Centre that supplies other growers with starting materials.

Major conclusions:

1. Farms are vary variable regarding structure, extend of own breeding and propagation. Bulk production with only a few varieties or on-farm developed special varieties can be profitable depending on expertise and strategy. Up-scaling of production is an important issues to reach large export markets (producing containers as an export unit).
2. Expertise for orchid growers is often developed as learning by doing and transferred within families. Innovations are much more started at farms than by institutional research. A passion for "colorful, trendy orchids" is clearly a target for the growers.
3. About 10% of the growers produce for export markets. Insecticide applications with alternating chemicals applied at a weekly base and a final methyl bromide fumigation is considered necessary to comply with the zero tolerance for export.
4. Traps are sometimes used to partly control or monitor insects but they are not used as a control thresholds. Biocontrol for insects is not applied and often the frequent colonisation by thrips "from outside" is mentioned
5. Plant-Health or Plant-Vigour is an issue that is appealing though most growers do not related that to disease or insect resistance.
6. Environmental awareness (nutrient / pesticide use or impact) did not automatically come up from the farmers in any discussion. Any drive to reduce seems to be absent. So incentives should come from quality improvement or cost reduction.