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Natural variation in herbivore induced plant volatile emission in ecotypes of *Arabidopsis thaliana*

No: 0809

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860904-145-010

October 2007-April 2008

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Abstract

Plants are constantly under attack by herbivorous insects. Indirect defense involves the participation of three trophic levels: the plant, the herbivore and a predator or parasitoid of the herbivore. After herbivore feeding the plant starts to emit *de novo* produced volatiles, so called herbivore induced plant volatiles (HIPVs). These HIPVs are used by predators or parasitoids and help them to locate the herbivore feeding on the plant. A difference can be observed between the quantity and quality of emitted HIPVs between plant species and even between plant cultivars and ecotypes. *Arabidopsis thaliana* from the family of *Brassicaceae* also emits HIPVs after herbivore feeding. The species has a large dispersal area which causes numerous ecotypes to exist, each having a complex genetic background. This genetic variation between ecotypes might be an indication of differences in HIPV blends of ecotypes. The main objective of this thesis is to investigate if genetic differences between Arabidopsis ecotypes responsible for HIPV emission are large enough to enable parasitoid *Diadegma semiclausum* to discriminate between the ecotypes. A two-choice ecotype test is performed in which the parasitoid can show its preference for one of the ecotypes. Six ecotypes of Arabidopsis, Kond, Eri, Cvi, Ws, Col0 and Ler are tested in 11 different combinations. Results of the experiments show that there are indeed several ecotype combinations that evoke a preference in the parasitoid. According to literature and the results of this thesis a rough picture about the importance of certain volatile groups can be drawn. Green leave volatiles and glucosinolates are not very important as HIPVs for *D. semiclausum*. Terpenoids are an important group of HIPVs as ecotypes which emit high quantities of terpenoids seem to be attractive.

1. Introduction

Plants are constantly under attack by all sorts of organisms, an important group of attackers are the insects: approximately half of all insect species are herbivore. As 80% of all animal species are insects, this is a serious threat. Insects cause a 10% reduction in above-ground plant parts of natural vegetation next to the damage sucking insects cause by removing essential compounds from the plant (Schoonhoven, 2005). To prevent or reduce damage by herbivorous insects, plants need to defend themselves. These defense responses result in an increase of fitness for the plant (Van Poecke and Dicke, 2004). Feeding or ovipositing by an herbivore induces a cascade of signal transduction pathways in the plant which involves gene expression that increases the level of specific hormones (Dicke *et al*., 2003). This defense can be direct or indirect (Van Poecke, 2007).

1.1 Direct plant defense

Direct defense is immediately directed towards the attacker, and can be divided into constitutive and induced direct defense. Constitutive direct defense is present in a plant without induction and includes both biochemical and morphological defenses. Morphological constitutive direct defenses are plant structures that make feeding on plant tissue less easy, like leaf-hairs (trichomes) or leaf waxes. Observations in the field have shown that *Arabidopsis thaliana* plants with trichomes experience less damage from herbivorous insects compared to hairless plants (Passardi *et al*., 2007). Especially flea beetles and moths are affected by trichomes (Van Poecke, 2007). Biochemical constitutive direct defense comprehend low concentration of toxins and feeding deterrents in plant tissue. These biochemicals retard the development and growth of an herbivore or reduce damage by preventing the herbivore to eat. The concentrations of these biochemicals might be raised after an herbivore damages plant tissue; this is a form of induced direct defense (Van Poecke, 2007). Inducible defenses can next to this biochemical defense also include morphological forms of defense. An example of this morphological inducible defense is the increased density and/or number of trichomes on newly formed leaves in a reaction to the damage of older leaves on the same plant. Many plant species apply this type of inducible morphological defense like Arabidopsis (Traw and Bergelson, 2003).

1.2 Indirect plant defense

Indirect defense occurs in a tritrophic context, which next to a plant and an herbivore also involves carnivorous insects like predators or parasitiods (Vet and Dicke, 1992). Arabidopsis is one of the plant species in which indirect defense has been reported. The induction of indirect defense begins with the raise of specific hormones that activates certain signal transduction pathways. The three most important signal transduction pathways are the octadecanoid pathway that involves jasmonic acid (JA), the shikimic pathway that involves salicylic acid (SA) and the ethylene pathway (Dicke *et al*., 2003). These increased hormone levels result in more gene expression, followed by the production and emission of *de novo* produced volatiles, so called herbivore induced plant volatiles (HIPVs) (Jervis and Kidd, 1996) These HIPVs attract carnivores like predators and parasitoids that can lead them to their prey or host (Van Poecke and Dicke, 2004).

1.2.1 Parasitoid host location

Parasitoids use chemical cues to locate their host, these cues can originate from the herbivore or from the herbivore infested plant. As there are many cues the parasitoid is exposed to, selecting the most useful one is of importance. To select useful cues, two factors are important: how reliable is the cue in indicating host presence and to what degree can this cue be detected. Cues derived from the host itself are most reliable. However, these cues are hard to detect over longer distances. Cues derived from plants like HIPVs are usually more easy to detect but less reliable to predict actual host presence. The best way to localize a host is to combine cues from both the plant and the host itself (Vet and Dicke, 1992). After a habitat is entered in which a possible host is present, the parasitoid starts searching more accurate to locate the host. Short-distance localization is mostly based upon chemical cues emitted by the host itself such as secretions from salivary glands, cuticular secretions (wax) or honeydew. These materials are deposited on the feeding substrate. (Jervis and Kidd, 1996) It seems most likely that parasitoids use easy to detect cues like plant volatiles to locate host habitats while host location is based upon host derived cues (Vet and Dicke, 1992).

1.2.2 HIPV production

After a plant is damaged by mechanical wounding or herbivore-feeding a complex of signaling cascades induces defense responses. One of these signaling cascades involves oxylipins which are acyclic or cyclic oxidation products derived from fatty acids (Creelman and Rao, 2002). JA is a good example, a linolenic acid-derived oxylipin produced by the octadecanoid pathway (Halitschke and Baldwin, 2005). The biosynsthesis of JA begins with linolenic acid (LA) which is converted into 13-hydroperoxylinolenate by lipogenases (LOX). After dehydration by an allene oxide synthase (AOS) an unstable allene oxide is formed. This unstable allene oxide is transformed by an allene oxide cyclase (AOC) into 12-oxo-phytodienoic acid (OPDA), a precursor of JA. To form JA out of OPDA, an OPDA reductase (OPR) and three ß-oxidation steps are necessary. During the ß-oxidation enzymes degrade fatty acids by removing two carbon units (Creelman and Rao, 2002). JA, together with OPDA, methyl jasmonate (MeJA) and conjugates are called jasmonates (JAs). JAs are one of the most important classes of signaling chemicals after wounding or herbivore-feeding. The activation of defense responses involves more oxylipins than just JAs which still need to be identified (Halitschke and Baldwin, 2005).
Once LOX converted LA into 13-hydroperoxylinolenate this compound can also be used by hydroperoxide lyase (HPL). HPL cleaves 13-hydroperoxylinolenate into a C_{12}-oxo-acid and a C_6-aldehyde, also called green leave volatiles (GVLs) (Creelman and Rao, 2002).
Van Poecke and Dicke studied the function of JAR1, an adenylate-forming enzyme for JA, forming isoleucine (JA-Ile). As mutants deficient in the JAR1-gene do not show a reduced attraction to natural enemies after herbivory, we can conclude that JA-Ile is not required for the production of HIPVs (Van Poecke and Dicke, 2003). Attractiveness to a natural enemy is reduced when JA biosynthesis is blocked in an early stage, which suggests that OPDA or another substance in the octadecanoid pathway acts as a signal chemical in HIPV induction (Halitschke and Baldwin, 2005).

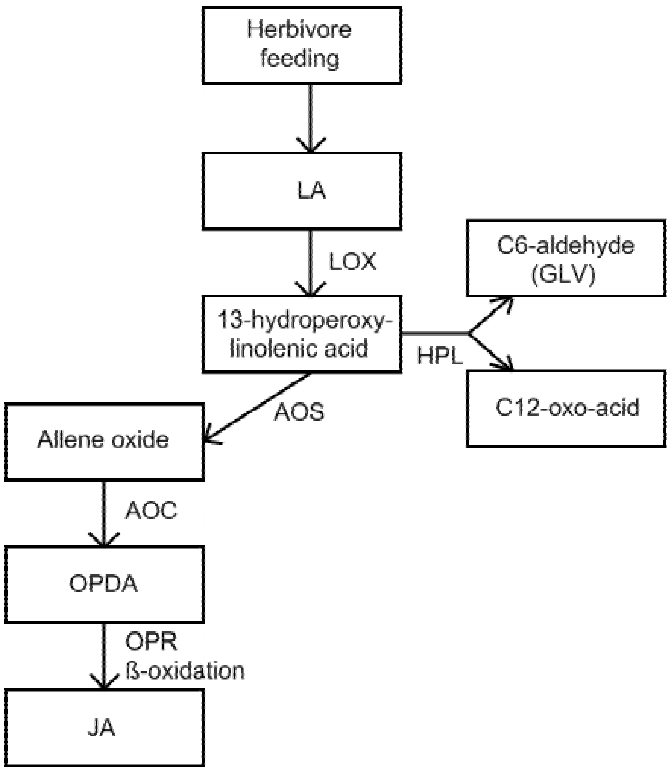

Figure 1. The biosynthesis pathway in which JA and GLVs are formed after herbivore feeding. The boxes are intermediate or end products of a reaction, the indications next to the arrows are proteins or reaction cycles catalyzing the particular reaction.

1.2.3 HIPV compounds

Common HIPVs are terpenoids, GLVs (C_6-aldehydes, -alcohols and -esters), phenolic compounds, nitrogenous compounds and aromatic compounds like methyl salicylate (MeSA) and indole (D'Alessandro *et al*., 2006).
Volatile terpenoids include monoterpenes (C_{10}), sesquiterpenes (C_{15}) and homoterpenes (C_{11} or C_{16}). Terpenoids act as volatiles and can activate JA-induced defense responses in neighboring plants. This might proof that terpenoids have a function in plant-plant interactions (Arimura *et al*., 2005 and Fäldt *et al*., 2002). Non-volatile terpenoids can mediate cell-to-cell signaling during induced plant defenses (Arimura *et al*., 2005). In Arabidopsis 40 genes have been identified that encode terpenoid synthases (AtTPSs). These enzymes are responsible for the synthesis of terpenoids (Fäldt *et al*, 2002). The AtTPS03 synthase belongs to the large AtTPS gene family and encodes for (E)-ß-ocimene, a monoterpene that is one of the most common released volatiles after herbivore feeding. JA treatment together with mechanical wounding caused accumulation of AtTPS03 transcripts in Arabidopsis leaves. As part of the HIPV blend emitted after herbivore damage, (E)-ß-ocimene can serve as an attractant to natural enemies of herbivores (Fäldt *et al*, 2002).
C_6-volatiles (GLVs) consist of 6 carbon volatiles like aldehydes, alcohols and esters. They are released directly after wounding of leaves. Arabidopsis leaves show already 20 seconds after wounding the formation of the first C_6-volatile compounds. As C_6-aldehydes and -alcohols reduce aphid fecundity on tobacco leaves, they are also suggested to play a role in direct defense against herbivorous insects (Arimura *et al*., 2005).
Indole is an aromatic compound formed by the shikimic acid pathway (D'Alessandro *et al*., 2006) and is sometimes observed as a compound in HIPV blends (Arimura *et al*., 2005)

Research was conducted in which the role of indole was tested in attracting parasitic wasps. Analysis showed that indole is a substantial part of the volatile blend that is emitted by herbivore infested maize plants. Two parasitic wasp species were offered volatile blends that included or lacked synthetic indole. One of the wasp species (*Cotesia marginiventris*) had no preference for either of the two blends, while the other species (*Microplitis rufiventris*) was deterred by the blend that included indole. Although indole is a substantial compound present in the emitted volatile blend of maize, it is not essential in the attraction of the two wasp species tested (D'Alessandro *et al*., 2006).
MeSA is the volatile form of salicylic acid (SA) (D'Alessandro *et al*., 2006) and can induce defense responses in plants (Arimura *et al*., 2005). MeSA is produced by the phenyl propanoid pathway, in which phenylalanine ammonia-lyase (PAL) is an essential enzyme (Van Poecke *et al.*, 2001). Treatment of plants with MeSA is effective against plant pathogens as defense signaling pathways are induced. However, MeSA treatment of plants has not been proven to enhance the attraction of carnivores (Shimoda *et al*., 2002).

Another group of secondary metabolites may be of importance in tritrophic interactions: the glucosinolates (GSs). GSs are herbivore defense compounds present in *Brassicaceae* that may be repellant or even toxic to insects. Some specialistic herbivore feeders however developed resistance against GSs and even use them as stimuli for feeding. Next to a direct defense action, volatile degradation products of GSs can also attract specialized parasitoids of *Brassicaceae* feeders (Van Poecke, 2007). The production of GSs and possibly the degradation products can be induced by herbivore feeding. Exogenous application of JA increases the quantity of GSs in plant tissue two times, especially the amount of the GS indolyl (Mewis et al., 2005). The degradation of GSs begins with the removal of glucose, further degradation is depending on the presence of a functional epithiospecifier protein (ESP). We can discriminate between two groups, one having a functional ESP and the other having a non-functional ESP. The epithiospecifier protein (ESP) catalyzes the hydrolysis of GSs into nitriles, when plants have a non-functional ESP, isothiocyanates are formed. Group one has predominantly isothiocyanates as end products, whereas group two has nitrile end products (Lambrix et al., 2001). Arabidopsis ecotypes from group one like Ler and Cvi have a functional ESP that gives predominantly nitrile end products, ecotypes from group two like Col0 and Ws have a non-functional ESP that gives predominantly isothiocyanates end products (Van Poecke, 2007). Especially isothiocyanates have showed to be toxic to insects. *Pieris rapae* caterpillars are able to form nitriles out of isothiocyanates with the help of a nitrile-specifier protein. This protein seems to be a highly sophisticated adaptation that allows the specialistic herbivore *P. rapae* to detoxify isothiocyanates (Wittstock et al., 2004). Also specialistic herbivore *Plutella xylostella* has adapted a system to feed on *Brassicaceae* without being affected by toxic GSs. *P. xylostella* has a glucosinolate sulfatase that largely blocks the formation of toxic hydrolysis compounds by the plant (Ratzka et al., 2002). Although the mechanisms are different, both insects are able to disarm the GS defense system of *Brassicaceae* species. Van Poecke and Dicke showed that *C. rubecula* can distinguish between Col0 plants mechanically damaged and infested with *P. rapae*. One of the compounds absent in mechanically damaged Col0 but present in infested Col0 is nitrile. Possibly *C. rubecula* uses this volatile GS degradation product to locate its host (Van Poecke and Dicke, 2003).

Induced volatile blends are very complex, already 1000 different chemicals have been identified, this number is still rising as more plants will be examined (Pichersky *et al*., 2006). The blend that is emitted by a plant differs with the herbivore species that is attacking the plant. The blends are qualitatively similar, but the ratios between the compounds are different.

Some carnivores are able to learn to discriminate between herbivore species by the volatiles that are emitted by the plant (Dicke *et al*., 2003).

1.2.4 Mechanical vs. herbivore damage

There are differences in the plant volatile blends after herbivore damage or mechanical damage as plants can discriminate between these two. Discrimination by the plant is made possible due to elicitors derived from oral secretions of the herbivore. These compounds can be all sorts of enzymes, glucose oxidase or fatty-acid amino-acid conjugates (FACs) in the regurgitant of the herbivore. If these elicitors are detected, plants know their tissue is damaged by an herbivore and not by mechanical damage (Dicke *et al*., 2003). Roda *et al* demonstrated that in the regurgitant of *Manduca sexta*, a specilistic herbivore of *Nicotiana attenuata* the two most abundant FACs cause a JA burst that leads to all measured direct and indirect defense responses in the plant. These FACs are necessary for the plant to recognize his attacker and induce a proper defense response (Roda *et al*., 2004). Different elicitors or elicitor combinations help the plant to identify the herbivore species and adapt the response according to this identification (Dicke *et al*., 2003).

In Lima bean, mechanical wounding causes local depolarization of the membrane at the site of damage. Infestation with a herbivore also induces membrane depolarization but also intracellular influx of calcium. Already with this first reaction to wounding there is a clear difference in signal transduction pathway when we compare mechanical wounding and herbivory. This indicates that both wounding and herbivore-specific elicitors are necessary to induce a complete defense response (Arimura *et al*. 2005).

In an experiment conducted by Conner *et al*. naïve *Cotesia glomerata* wasps were used in a wind-tunnel bioassay to compare the attractiveness of Brussels sprout plants damaged by herbivores, mechanically damaged by a single event and repeated mechanical damage. After 8 hours of damage, both herbivore damage and repeated damage were preferred over single event damage and *C. glomerata* did not significantly discriminate between repeated and herbivore damage. From this we can conclude that repeated infliction of damage induces a plant response similar to herbivore damage. Single mechanical damage however elicits a plant response that is phenotypically distinct from herbivore damage and enables a parasitoid to discriminate between the two (Conner *et al*. 2007).
In tobacco cDNA was isolated after mechanical damage that encodes a protein kinase (WIPK) that is considered essential for JA formation. Mutants in which WIPK is silenced show lower concentrations of JA after wounding. This might indicate that WIPK is an early activator of the octadecanoid pathway (Arimura *et al*., 2005).

1.2.5 Research on single gene function

The emission of HIPVs can be manipulated to improve the natural defenses of crop plants. For this manipulation knowledge is required about the genes that are responsible for the biosynthesis of HIPVs together with insight into the complex regulatory networks of HIPV emission (Dudareva and Negre, 2005). One way to do it, is to investigate the role of single genes or small groups of genes. Recent developments in genomics and ecology can be combined to provide tools that can be used to perform these experiments. One of these tools is creating mutants that differ in only one gene. With these genotypes, experiments can be conducted to unravel the role of this single gene in induction of plant volatiles. Another way of identifying genes involved in the production of HIPVs is to use transcriptomics and

metabolomics. Gene expression profiles and data of volatile production can be combined to reveal involvement of the studied genes in the production of HIPVs (Snoeren *et al*., 2007).

Plant responses to herbivore feeding can be monitored by comparing gene-expression of undamaged plants with that of damaged plants. Van Poecke *et al*. did this for Arabidopsis plants damaged by *P. rapae*. Caterpillar feeding induced the expression of three lipoxygenase genes: *AtLOX2, AtHPL and AtAOS,* two genes involved in terpenoid biosynthesis: *AtTPS03, AtTPS10* and one gene encoding PAL: *AtPAL1*. The expression of these genes was in good correlation with the emission of volatiles after herbivore induction (Van Poecke *et al*., 2001).

1.2.6 Interaction between above- and belowground tritrophic systems

In a situation where one host plant is attacked by above- and belowground herbivores at the same time the resistance in both systems may be affected. Also indirect defenses may be influenced as primary and secondary metabolites need to be allocated in a different way. The tritrophic interaction for aboveground systems was recently also found to exist for the belowground system (Rasmann and Turlings, 2007). Masters *et al*. showed that root herbivory increased the attraction of parasitoids which is probably caused by the increased nutritional quality of the host plant which makes it more attractive to the parasitoids host (Masters *et al*, 2001). Negative effects of root feeding on aboveground interactions have also been reported, like a change in parasitoid foraging behavior due to changes in HIPV blend. After insect feeding damage, roots of maize emit the sesquiterpene (E)-β-caryophyllene which attracts insect feeding (entomopathogenic) nematodes. Rasmann and Turlings performed a study in which at the same time an aboveground and a belowground tritrophic system were tested in an olfactometer. The interactions of above- and belowground tritrophic systems add another factor in the already complex network of indirect plant defense (Rasmann and Turlings, 2007).

1.2.7 Costs and benefits

Benefits of indirect plant defense seem obvious, damage can be reduced when carnivores can be effectively attracted to eat or parasitize the herbivore (Van Poecke and Dicke, 2004). Plants benefit of predation seems obvious, the herbivorous insect is immediately removed from the plant and can cause no further damage, and this is different for parasitism. A parasitized insect is not removed from the plant but remains feeding until it pupates. Damage reduction does not necessarily mean increased plant fitness. Van Loon *et al*. show that Arabidopsis plants infested with parasitized caterpillars of *P. rapae* did not suffer from a significantly reduced seed production compared to control plants. This in contrast to Arabidopsis plants infested with unparasitized caterpillars, these plants showed a significant reduction in seed production compared to control plants (Van Loon *et al*., 2000). Next to the benefits it is also important to check the costs of indirect plant defense. Volatile blends cause the plant to stand out more to other members in the environment. In an environment in which large numbers of carnivores are present, the net result of emitting volatiles may be positive. However, in an herbivore-rich environment the net outcome may turn out negative. So the costs and benefits for indirect plant defense may be largely dependent upon the ecological context (Dicke *et al*., 2003). The overall cost or benefit of emitting volatiles can be measured by plant fitness like seed number, and weight (Van Poecke and Dicke, 2004). Investment of energy in defense means that this energy can not be used in growth and development. The consideration whether or not to emit volatiles is a trade-off that plants have to make constantly to reach maximum fitness (Dicke *et al*., 2003).

1.2.8 The effect of genetic variation on HIPV blends

As plants differ in their genetic background and environment, a difference can be expected in the volatile blend composition. A quantitative difference in volatile production indicates that the composition of the blend is similar, but the amount in which it is produced or the ratio's between the components are different. A qualitative difference in volatile blend indicates that the compounds in the blend are different (Vet and Dicke, 1992). Differences can be observed in quantity and quality of HIPVs between plant species and even between cultivars of the same species (Krips et al., 2001). These differences might be explained by genetic variation (Gouinguené et al., 2001). The variation in volatile composition has been observed in different plant species like maize, gerbera, cotton and Arabidopsis. These differences in volatile blends make it for natural enemies even more difficult to find a suitable prey or host with the help of volatiles. Despite this complexity, there are examples which show that natural enemies are capable of distinguishing between volatiles of closely related plant cultivars (Vet and Dicke, 1992).

Variation in volatile emission is to a large degree caused by genetic differences as we see in a study performed in by Degen *et al*. (2004). In this study, maize is used to get insight in the genetic variability of HIPV emission within inbred lines. The 31 inbred lines used in this study were genetically related but showed a broad range of genetic diversity. The production of HIPVs was induced by injecting the maize plants with caterpillar regurgitant which showed a surprisingly similar odor pattern compared to caterpillar infestation for each line. Of the 23 most abundant volatile compounds emitted, there were 17 terpenoids present. Both the quantitative and qualitative differences of emitted HIPVs varied largely between the inbred lines. This variation is to a large degree caused by genetic differences among the lines (Degen *et al*. 2004).Gouinguené *et al*. compared volatiles emitted by maize cultivars and wild maize species after mechanical damage to the leaves and applying oral secretions of *Spodoptera littoralis*. In this way they try to get insight into the genetic factor involved in volatile emission. The quantitative amount of volatiles differed among the genotypes tested. Between the wild species there was a significant difference in the total amount of volatiles emitted. Some genotypes released 8 times more volatiles than others. Also a qualitative difference could be observed, each genotype had its own characteristic volatile blend. Differences were found in the compounds present in the blend and the ratio between these compounds. Especially three sesquiterpenes including ß-caryophyllene varied largely between the genotypes tested. Another remarkable difference between the genotypes tested is the time that ecotypes need between damage and volatile emission and the emission peaks after that. In maize the odor blends of cultivated maize contains the same compounds as the wild species (Gouinguené et al., 2001).

Krips et al. compared the attractiveness and composition of HIPV blends of four cultivars of *Gerbera jamesonii.* They infested the cultivars with spider mite *Tetranychus urticae* and investigated the attractiveness of the emitted HIPVs to specialistic predatory mite *Phytoseiulus persimili*s. One of the cultivars was less attractive compared to two of the other cultivars. When the HIPV blends of the cultivars were analyzed the less attractive cultivar showed to emit less than 50% of the total amount of HIPVs. Also the total quantity of terpenoids was lower in this cultivar compared to the others (53% compared to 70-85%). Possibly the quantity of the total volatile blend together with the quantity of terpenoids in the blend explain the attractiveness to predatory mite *Phytoseiulus persimili*s (Krips et al., 2001).

1.2.9 Arabidopsis ecotypes

As a plant species has a large dispersal area, it is to be expected that many different habitats are occupied. To have a similar fitness in all of these different environments, plants need to adjust themselves to the local conditions. The result is a difference in physiological and morphological characteristics. These differences usually have a complex genetic basis in which sometimes hundreds of genes are involved. These differences result in distinct groups that are called ecotypes (Raven et al., 2003). *Arabidopsis thaliana* is a widespread species (Fig. 2), so it is not surprising that there are many Arabidopsis ecotypes. More than 750 Arabidopsis ecotypes have been collected so far, from which the seed is stocked in large seed stock centers (Passardi *et al*., 2007). As a mostly self-pollinating species, Arabidopsis ecotypes are wild homozygous lines (Koornneef *et al*., 2004). Already a lot is known about the specific traits of some Arabidopsis ecotypes, this knowledge makes it possible to select ecotypes with the traits of interest.

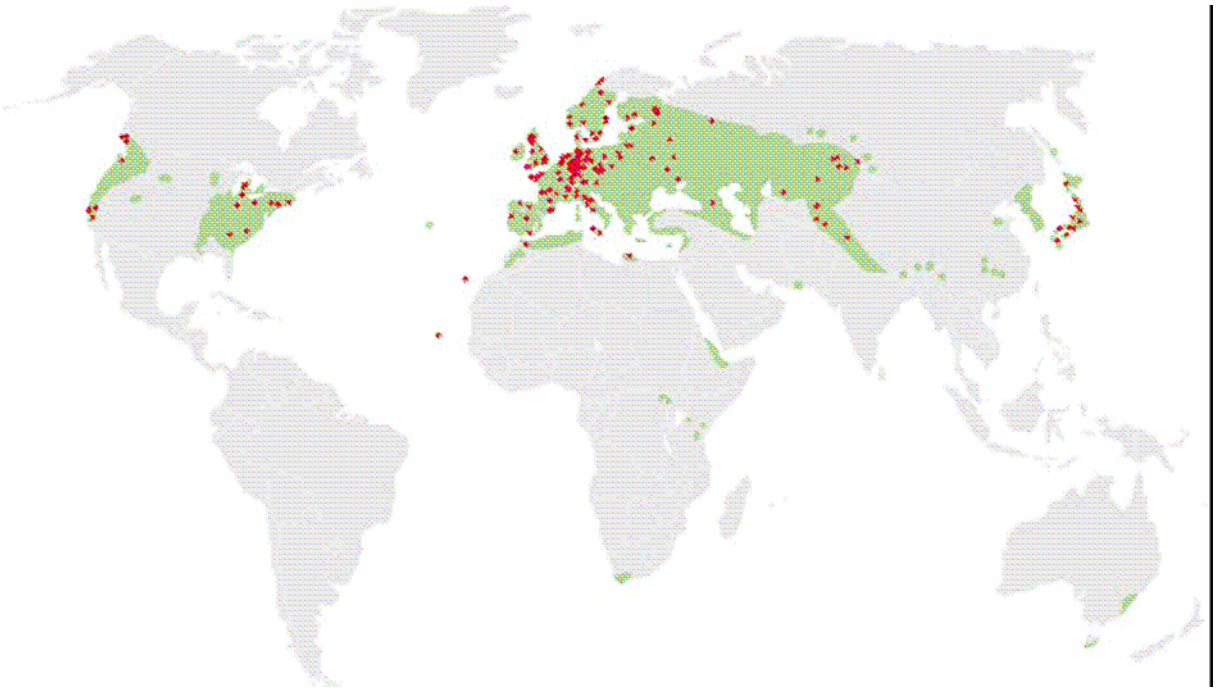

Figure 2. The green area covers the dispersal area of *Arabidopsis thaliana* worldwide, the red dots represent the places from which different ecotypes were collected (Koornneef *et al*., 2004) .

Ecotypes can be selected based on certain traits that seem interesting for further research. Volatile blends have a large influence on the attraction of parasitoids, as this is a very important way of plants and parasitoids to communicate. That is why it is interesting to select ecotypes with different volatile blends to test them against each other and study the effect on the carnivore.

1.2.10 Genetic variation and HIPV blends in Arabidopsis ecotypes

Arabidopsis ecotypes that originate from different locations show phenotypic variation that is caused by genetic variation naturally occurring in the species. There is a wide variation in morphological and physiological traits between ecotypes. Using this natural genetic variation the function of individual genes can be studied. Although mutants have been extensively used for this research, the little amount of analyzed genetic backgrounds is limiting the identification of individual gene function. The ecotypes Landsberg erecta (Ler) and Columbia

(Col0) contain large numbers of gene deletions and documentation of loss of functions in other ecotypes is enlarging. In ecotype Ler, a high number of Col0 genes were found to be deleted of which several encoded secondary metabolites (Koornneef *et al*., 2004). Duan *et al*, studied transcripts of the CYP74B2-gene in ecotypes Ler and Col0 that encode the HPL protein involved in the production of GLVs. In Col0 these transcripts were expressed at a significantly lower level compared to Ler. The cause is a deletion in the CYP74B2-gene in Col0. Other transcripts expressed at a lower level in Col0 compared to Ler encode LOX2, LOX3, glucosinolates and JAs like OPDA reductase (OPR3). Compared to Ler, leaves of Col0 plants contained a reduced amount of hexanal and no trans-2-hexanal (Duan *et al*., 2005). Studying natural genetic variation also helps to understand mechanisms that generate and maintain this variation. These mechanisms might involve adaptation to environmental factors and will provide an ecotype with ecological advantages. The advantages of maintaining certain phenotypic traits might not always be clear, in this case the trade-offs between different traits must be studied to create a clear image. Probably the easiest way to identify natural genetic variation in ecotypes is direct comparison. For other traits, this way of identification is not possible, in these cases ecotypes need to be crossed and the offspring need to be checked for displaying a phenotype outside the parental range (Koornneef *et al*., 2004).

Tholl *et al*. investigated volatiles emitted by Arabidopsis flowers. The volatile blend exists largely out of a group of 20 sesquiterpenes which are almost all formed by two terpene synthases encoded by the genes At5g23960 and At5g44630. After screening of 37 Arabidopsis ecotypes there were quantitative but only little qualitative differences detected in the floral volatile blend. This is an indication that volatile monoterpenes and sesquiterpenes are essential for Arabidopsis survival (Tholl et al., 2005).

1.2.11 HIPV research

To gain a better understanding of the relationship between HIPVs and parasitoid behavior, it is essential to have some distinct lines that differ in the quantity or quality of HIPV emission. For Arabidopsis there is an enormous number of mutants available, these mutants originate from different ecotypes. As these mutants are often used in all sorts of scientific research, the natural variation between ecotypes could influence the conclusions that are drawn according to experiments conducted with these mutants. This is why it is interesting to demonstrate if there is natural variation in HIPV emission between ecotypes that enables parasitoid wasps to discriminate between these ecotypes. Already much research is done on the genetic differences between Arabidopsis ecotypes, including genes involved in the production and induction of HIPVs. Duan *et al*. and Borevits *et al*. showed the genetic differences between ecotype Ler and Col0 (see previous section). Tholl *et al*. indicated that ecotype Cape Verde Islands (Cvi) emits no sesquiterpenes, which are important floral volatiles (Tholl *et al*., 2005). On the other hand Cvi has a strong induction of JA when compared to ecotype Wassilewskija (Ws) (Kuśnierczyk *et al*., 2007). According to this knowledge we can select ecotypes that differ in volatile emission that influence parasitoid host finding.

As described before, JA plays an important role in the production of volatiles after herbivore feeding. Feeding damage can be simulated by spraying the plant with a JA-solution, although plants with herbivore infestation are more attractive to parasitoids compared to JA treated plants which indicates that not only JA is involved in volatile production. Results of bioassays conducted with Lima bean plants show that the volatile blends emitted by herbivore infested and JA-treated plants are similar but not identical. Parasitoids also confirm this by choosing

herbivore infested plants over JA-treated plants in a Y-tube olfactometer test (Dicke et al., 1999). Treatment of *Arabidopsis thaliana* plants with JA also results in HIPV production and attraction of parasitoids (Van Poecke and Dicke, 2004). Comparing different combinations of ecotypes in a two-choice experiment can give a clear image of the preferences of the parasitic wasps used.

Studying genetic variation naturally occurring in a common species like Arabidopsis is interesting from an evolutionary point of view. One way to study this natural genetic variation is comparing HIPV-blends emitted by different ecotypes. This comparison can be made by inducing HIPVs and testing their attractiveness to a parasitoid in a two-choice experiment. A combination can be made between two ecotypes in which a possible preference for a certain ecotype might be detected. This preference might give an indication if the genetic differences mentioned in literature are sufficient for the parasitoid to discriminate between the two HIPV-blends of the ecotypes.

Can parasitic wasp *Diadegma semiclausum* discriminate between HIPVs emitted by different Arabidopsis ecotypes when these are compared in a two-choice experiment?

2. Materials and methods

2.1 Plant material

2.1.1 Ecotype selection

During the experiment we will use six different *Arabidopsis thaliana* ecotypes: Wassilewskija (Ws), Cape Verde Islands (Cvi), Kondara (Kond), Eriengsboda (Eri), Columbia (Col0) and Landsberg erecta (Ler). They respectively originate from Russia, Cape Verde Islands, Tadjikistan, Sweden, USA and Poland (Paulo *et al*., 2008; http://arabidopsis.org/). Kuśnierczyk *et al*. compared ecotype Ws, Ler and Cvi and demonstrated that Cvi and Ler lower the fitness of a specialistic herbivore feeder whereas Ws did not. This might be an indication that Ws has a lower production or induction of JA which makes it interesting to investigate. After herbivore infestation, Cvi had a strong up-regulation of AOS and LOX which can cause a raise in emission of GLVs. Ler had a strong induction of LOX2 after infestation. After infestation, Ws showed the highest number of up-regulated transcripts whereas Ler showed the lowest. The number of genes up-regulated at least two times compared to uninfested plants was highest in Cvi. Cvi and Ws showed highest similarity between the number of up- and down-regulated genes, whereas Cvi and Ler showed the lowest similarity. The strongest induction of the JA-synthesis pathway after herbivore infestation is found in Cvi (Kuśnierczyk *et al*., 2007). Col0 contains a deletion in the *CYP74B2*-gene which causes HPL transcripts to be expressed at a significantly lower level. These transcripts are involved in the production of C6-volatiles (green leaf volatiles), jasmonates and glucosinolates, all involved in induced direct or indirect defense (Duan *et al*., 2005). As ecotype Col0 emits very low amounts of green leave volatiles, it is to be expected that attraction of carnivores will not be so successful. Ecotype Cape Verde Islands (Cvi) emits no sesquiterpenes, an important group of floral volatiles (Tholl *et al*., 2005), but shows a strong induction of the JA signal transduction pathway compared to ecotype Ws (Kuśnierczyk *et al.,* 2007).

Ecotype	Phenotype
Ws	- Low production or induction of JA compared to Cvi (Kuśnierczyk *et al*., 2007). - After herbivore infestation, high number of up-regulated transcripts compared to Cvi and Ler (Kuśnierczyk *et al*., 2007). - Reduced GSs levels compared to Col0 (Cipollini *et al*., 2004).
Col0	- Incomplete HPL protein, no GLVs (Duan *et al*., 2005). - Reduced LOX2, LOX3, JAs and glucosinolates (Duan *et al*., 2005). - Reduced amounts of hexanal and no trans-2-hexanal (Duan *et al*., 2005).
Cvi	- No sesquiterpene emission (Tholl *et al*., 2005). - Strong JA induction compared to Ws and Ler (Kuśnierczyk *et al*. 2007). - Cvi emits nitriles (Van Poecke, 2007) which have showed to be attractive to *C. rubecula* (Van Poecke and Dicke, 2003).
Ler	- Absence of certain secondary metabolites (Koornneef *et al*., 2004) - Full length HPL protein, GLV emission (Duan *et al*., 2005)
Kond	- Preliminary headspace analysis reveal that 15 compounds are more than 2-fold more abundant in the induced volatile blend of Kond compared to the blend of Eri, these are mainly terpenoids (Snoeren, pers. com.).
Eri	- Compared to Kond, very little amount of certain terpenoids in HIPV-blend (Snoeren, pers. com.)

2.1.2 Soil

Potting soil used for the planting is special soil for growing Arabidopsis with 20% sand, no extra fertilizer is added. The soil is sieved and heated at 90ºC for 4 hours in an autoclave and put to rest for one week before use.

2.1.3 Ecotype growing

Arabidopsis plants are grown from seed using a plastic tray with 104 holes distributed over 8 rows and 13 columns. As the tray is filled with treated Arabidopsis soil as described above, the tray is placed in a container filled with water to evenly moist the soil. The seeds are obtained from stock material stored in a refrigerator. Seeding is done with the help of a wet brush or toothpick. The containers are placed in a climate room to germinate (23±1 ºC, 50-70% RH, 10 Klux, L8:D16). After 1,5-2 weeks, the individual plants are transferred to larger pots to promote growth. These pots are 7 cm of diameter and filled with treated Arabidopsis soil. After 5-6 weeks the plants are still in the vegetative state and are large enough to test. The average rosette diameter is 9 centimeters. The soil is dampened three times a week, in between the top layer of the soil dries in.

2.1.4 Nematode application

Infestation of the plants by larvae of the sciarid fly or fungus gnat *Bradysia agrestis* causes great stress on the plants as the larvae consume large parts of plant roots. Studies have shown that application of the entomophagous nematode species, *Steinernema carpocapsae* significantly reduced the number of sciarid fly larvae compared to control plants without nematode application (Kim *et al*. 2004).
To avoid or cure larval infestation in the Arabidopsis plants entomophagous nematodes are applied twice a week. The entomophagous nematodes *Steinernema feltiae* (ENTONEM, Koppert BV.) are stored in inert carrying material. Per application ¼ of the package is soaked in 1 liter of water at room temperature. The solution is stirred for 30 minutes at 200 rpm to gently activate the nematodes. As the number of plants present in the compartment changes over time the volume applied varies between 1 and 2 ml per plant, put directly on the soil after watering.

2.2 Plant treatment

As we work with different ecotypes, herbivores might feed differently on each ecotype, this might influence the amount of volatiles emitted possibly effect the outcome of the experiment. To avoid this effect of differences in consumed leaf tissue, we use JA treatment to standardize the HIPV-production. Control plants will be sprayed with a 0.1% Tween solution, except for the spraying they are treated in exactly the same way as the JA-treated plants. JA-treated plants will be sprayed with a 1mM solution of JA + 0.1% Tween to simulate infestation by an herbivore. The plants are sprayed in groups of four with approximately 1ml per plant of a 1,0 mM JA (Sigma-Aldrich) solution 24 hours before the plants are used in the experiment.

2.3 Insects

The parasitic wasp species *Diadegma semiclausum* is used, *D. semiclausum* is a specialized parasitoid of the generalist diamondback moth *Plutella xylostella*. *D. semiclausum* is reared

on *P. xylostella* feeding on Brussels sprouts under greenhouse conditions (20±2 ºC, 50-70% RH, L16:D8). The wasps get the opportunity to parasitize their host. After the host is parasitized, they are removed and transferred to a gauze cage in a climate room (23±1 ºC, 50-70% RH, L16:D8) in the absence of wasps and plants. Wasps that emerge from the parasitized hosts are fed *ab libitum* water and honey. In the rearing cage, males are allowed to mate with the females. Before the experiment the parasitic wasps are sexed, as only mated female wasps of 5-10 days of age will be used. These females are sensitive to volatiles emitted by the plants when they are ready to oviposit. As the *D. semiclausum* females have no oviposition experience they are referred to as naïve (Bukovinszky *et al.,* 2005). Female parasitic wasps are caught from the rearing cage and put in an experimental cage. The number of female wasps caught depends on the number of experiments that will be conducted during the week, to assure that all females used in the experiments are mated, 5-10% males are present in the experimental cage. The wasps are transferred to the experimental room one hour in advance of the experiment to allow adjustment to the changing conditions.

2.4 Y-tube olfactometer bioassay

The Y-tube olfactometer is a commonly used tool to study responses of parasitoids to olfactory stimuli (Bukovinszky *et al*., 2005). To test the responses of the female *D. semiclausum* wasps a glass Y-tube is used. This tube is Y-shaped and consists out of two arms that are connected to the base by a junction. Each arm is connected to a 5 liter glass container, between the container and the arm of the Y-tube there are two sieves put separated by a 10 cm glass pipe to prevent the wasps from entering the container. In the containers four plants of each treatment are placed. The containers are sealed air-tight so the plant odors stay inside. Air is filtered over charcoal and pumped into the containers through a plastic hose with 4 l/min, the airflow is measured with an airflow-meter. The base of the Y-tube is sealed by a rubber plug through which the air is extracted from the Y-tube with 8 l/min. Above the Y-tube, strong lights are used to distribute light evenly over the experimental set-up (Bukovinszky *et al*., 2005).

The female wasps are individually tested and used only once. They are gently caught from the cage with a small glass tube to reduce stress to the minimum. The wasps are placed together with the glass tube at the base of the Y-tube at 4 cm from the end. From here they get about 1 minute to leave the glass tube and enter the Y-tube. The wasps will walk or fly upstream towards one of the odor sources. As soon as the wasp leaves the glass tube she has 5 minutes to reach the junction of the Y-tube and make a first choice, if not; the wasp is not-responding and removed from the Y-tube. In total, the wasp has 10 minutes to reach the finish line 1 cm before the sieve at the end of each arm. If the wasp crosses the finish line and stays in the same arm for more than 15 seconds, this is considered as a choice for the particular odor source (Bukovinszky *et al.*, 2005). The Y-tube olfactometer bioassays will preferably be performed around noon as the wasps fly best around that time of the day, the temperature is kept constant around 22 ± 1ºC. The containers are switched from one arm of the Y-tube to the other to control for possible asymmetry in the experimental set up. Per experimental day two ecotypes are tested in the below mentioned combinations, the order in which the combinations are performed may differ:

Ecotype A blanc vs. Ecotype A JA: 6 wasps
Ecotype B blanc vs. Ecotype B JA: 6 wasps
Ecotype A JA vs. Ecotype B JA: 12 wasps

After each experiment the aboveground plant parts are removed and weighed, if the wasps consequently prefer ecotypes with larger biomass this might give a wrong image about HIPV emission.

3. Results

3.1 Ecotype combinations

In figures 3-13 the results of the Y-tube olfactometer bioassay are given. The results for each experiment are gathered over four or three experimental days. The figures show the odor source combinations made and the bars indicate the percentage of parasitic wasps choosing one of the odor sources. JA stands for the particular ecotype sprayed with 1 mM JA + 0.1% Tween solution, bl. is the abbreviation for blank and indicates the treatment of the particular ecotype with 0.1% Tween solution (also referred to as control treatment). N is the number of parasitic wasps that responded during the experiments. NR is the percentage of parasitic wasps not responding during the experiment.
As a statistical tool a two-sided binomial test was used, $P \geq 0.05$ is not significant; $P < 0.05$ is significant.

Experiment 1: Kondara vs. Eriengsboda

Do ecotypes Kond and Eri have a natural variation in volatile production that is relevant for parasitic wasps to discriminate between these ecotypes?

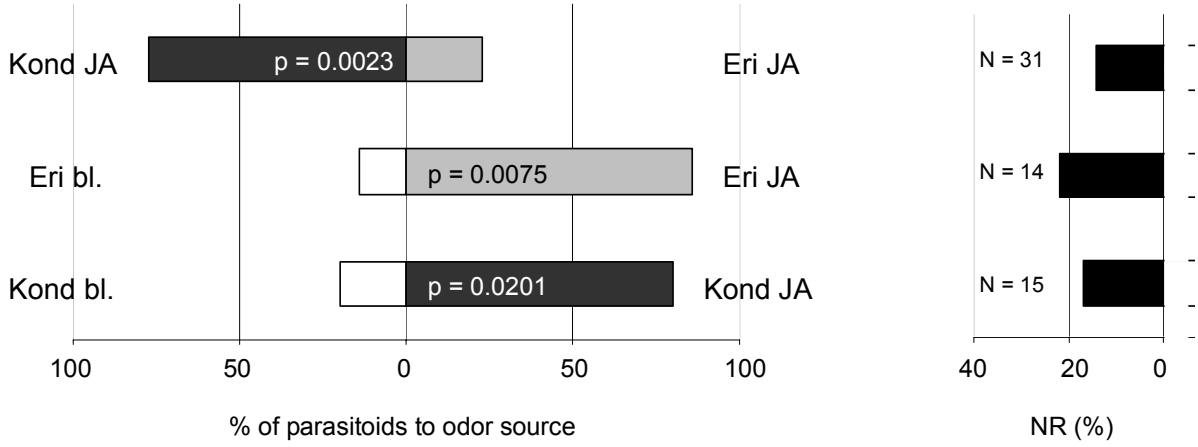

Figure 3: Response of *D. semiclausum* to Arabidopsis plants of ecotype Kond and Eri sprayed with JA or 0.1% Tween solution (bl.) in a Y-tube olfactometer bioassay. N is the number of wasps tested, NR is the number of wasps that were not responding. Choices between odor sources were analyzed with a two-sided binomial test, p-values are indicated in bars.

Results are gathered over three experimental days. Figure 3 shows that both Kond and Eri are more attractive to *D. semiclausum* when sprayed with JA compared to the control treatment. Volatiles emitted by Kond and Eri after induction by JA enable *D. semiclausum* to discriminate between JA and control plants. When comparing the JA-treatments of both ecotypes, Kond-JA is significantly more attractive than Eri-JA. So volatiles emitted by Kond and Eri after induction by JA vary in such a way that *D. semiclausum* is able to make a discrimination between the two ecotypes.

The average fresh weight of the leaves is in grams for Kond: JA 0.75 and bl: 0.80, for Eri: JA 0.6 and bl: 0.58.

Experiment 2: Kondara vs. Cape Verde Islands

Do ecotypes Kond and Cvi have a natural variation in volatile production that is relevant for parasitic wasps to discriminate between these ecotypes?

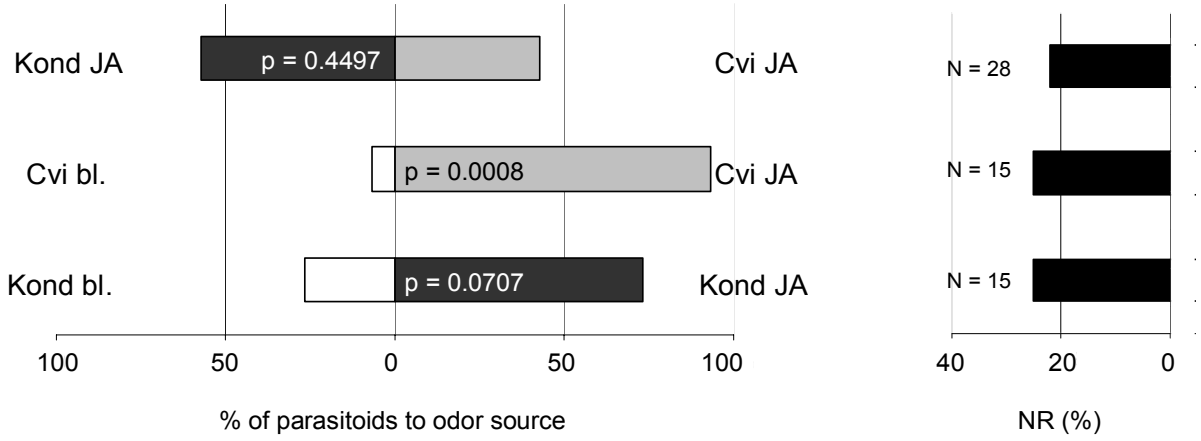

Figure 4: Response of *D. semiclausum* to Arabidopsis plants of ecotype Kond and Cvi sprayed with JA or 0.1% Tween solution (bl.) in a Y-tube olfactometer bioassay. N is the number of wasps tested, NR is the number of wasps that were not responding. Choices between odor sources were analyzed with a two-sided binomial test, p-values are indicated in bars.

Results are gathered over three experimental days. Figure 4 shows that only Cvi is significantly more attractive to *D. semiclausum* when sprayed with JA compared to the control treatment. Kond is slightly more attractive when sprayed with JA. Volatiles emitted by Cvi after induction by JA enable *D. semiclausum* to discriminate between JA and control plants. None of the two ecotypes is significantly more attractive when the JA-treatments are tested against each other. The volatiles emitted by Kond and Cvi after induction by JA are not relevant for *D. semiclausum* to discriminate between the two ecotypes.

The average fresh weight of the leaves is in grams for Kond: JA 0.66 and for bl 0.57, for Cvi: JA 0.55 and bl 0.52.

Experiment 3: Eriengsboda vs. Wassilewskija

Do ecotypes Eri and Ws have a natural variation in volatile production that is relevant for parasitic wasps to discriminate between these ecotypes?

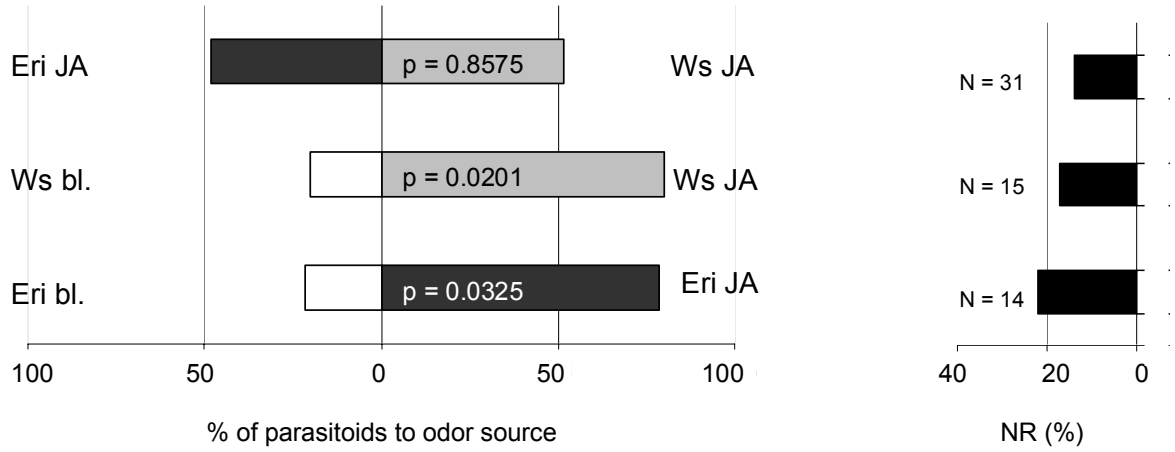

Figure 5: Response of *D. semiclausum* to Arabidopsis plants of ecotype Eri and Ws sprayed with JA or 0.1% Tween solution (bl.) in a Y-tube olfactometer bioassay. N is the number of wasps tested, NR is the number of wasps that were not responding. Choices between odor sources were analyzed with a two-sided binomial test, p-values are indicated in bars.

Results are gathered over three experimental days. Figure 5 shows that both Eri and Ws are significantly more attractive to *D. semiclausum* when sprayed with JA compared to the control treatment. Volatiles emitted by Eri and Ws after induction by JA enable *D. semiclausum* to discriminate between JA and control plants. None of the two ecotypes is significantly more attractive when the two JA-treatments of both ecotypes are tested against each other. The volatiles emitted by Eri and Ws after induction by JA are not relevant for *D. semiclausum* to discriminate between the two ecotypes.

The average fresh weight of the leaves is in grams for Eri: JA 0.76 and for bl 0.71, for Ws: JA 0.83 and bl 0.71.

Experiment 4: Wassilewskija vs. Cape Verde Islands.

Do ecotypes Ws and Cvi have a natural variation in volatile production that is relevant for parasitic wasps to discriminate between these ecotypes?

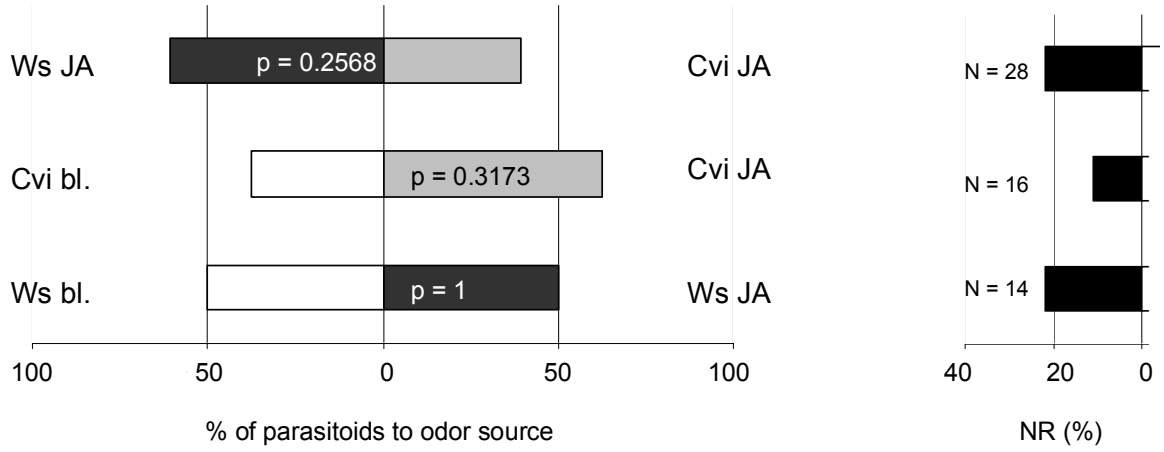

Figure 6: Response of *D. semiclausum* to Arabidopsis plants of ecotype Ws and Cvi sprayed with JA or 0.1% Tween solution (bl.) in a Y-tube olfactometer bioassay. N is the number of wasps tested, NR is the number of wasps that were not responding. Choices between odor sources were analyzed with a two-sided binomial test, p-values are indicated in bars.

Results are gathered over three experimental days. Figure 6 shows that neither Ws nor Cvi are significantly more attractive to *D. semiclausum* when sprayed with JA compared to the control treatment. Volatiles emitted by both Ws and Cvi after induction by JA do not enable *D. semiclausum* to discriminate between JA and control plants. None of the two ecotypes is significantly more attractive when the JA-treatments are tested against each other. The volatiles emitted by Ws and Cvi after induction by JA are not relevant for *D. semiclausum* to discriminate between the two ecotypes.

The average fresh weight of the leaves is in grams for Ws: JA 0.48 and bl 0.31, for Cvi: JA 0.51 and bl 0.36.

Experiment 5: Eriengsboda vs. Cape Verde Islands.

Do ecotypes Eri and Cvi have a natural variation in volatile production that is relevant for parasitic wasps to discriminate between these ecotypes?

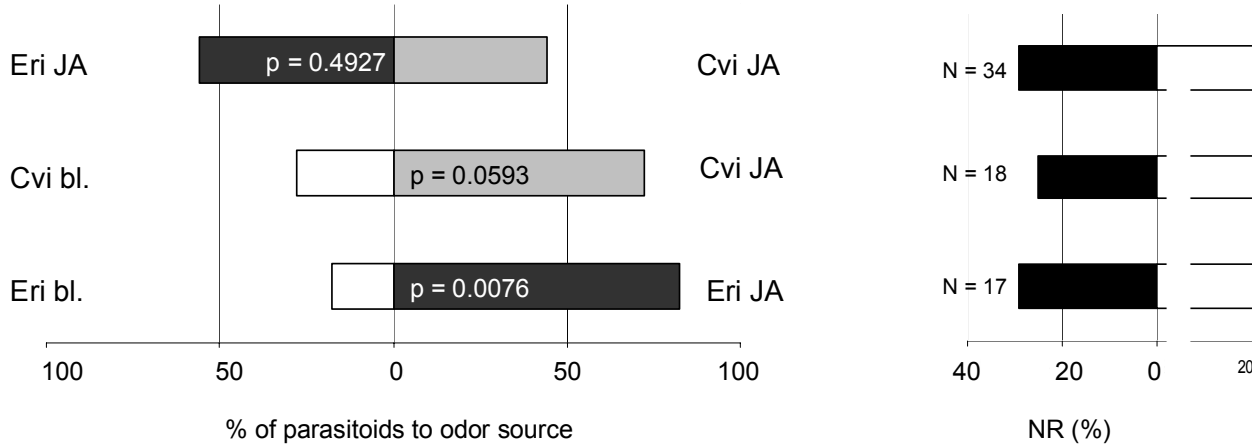

Figure 7: Response of *D. semiclausum* to Arabidopsis plants of ecotype Eri and Cvi sprayed with JA or 0.1% Tween solution (bl.) in a Y-tube olfactometer bioassay. N is the number of wasps tested, NR is the number of wasps that were not responding. Choices between odor sources were analyzed with a two-sided binomial test, p-values are indicated in bars.

Results are gathered over four experimental days. Figure 7 shows that only Eri is significantly more attractive to *D. semiclausum* when sprayed with JA compared to the control treatment. Cvi is slightly more attractive when sprayed with JA. Volatiles emitted by Eri after induction by JA enable *D. semiclausum* to discriminate between JA and control plants. None of the two ecotypes is significantly more attractive when the JA-treatments are tested against each other. The volatiles emitted by Eri and Cvi after induction by JA are not relevant for *D. semiclausum* to discriminate between the two ecotypes.

The average fresh weight of the leaves is in grams for Eri: JA 0.82 and bl 0.91, for Cvi: JA 0.68 and bl 0.65

Experiment 6: Kondara vs. Wassilewskija.

Do ecotypes Kond and Ws have a natural variation in volatile production that is relevant for parasitic wasps to discriminate between these ecotypes?

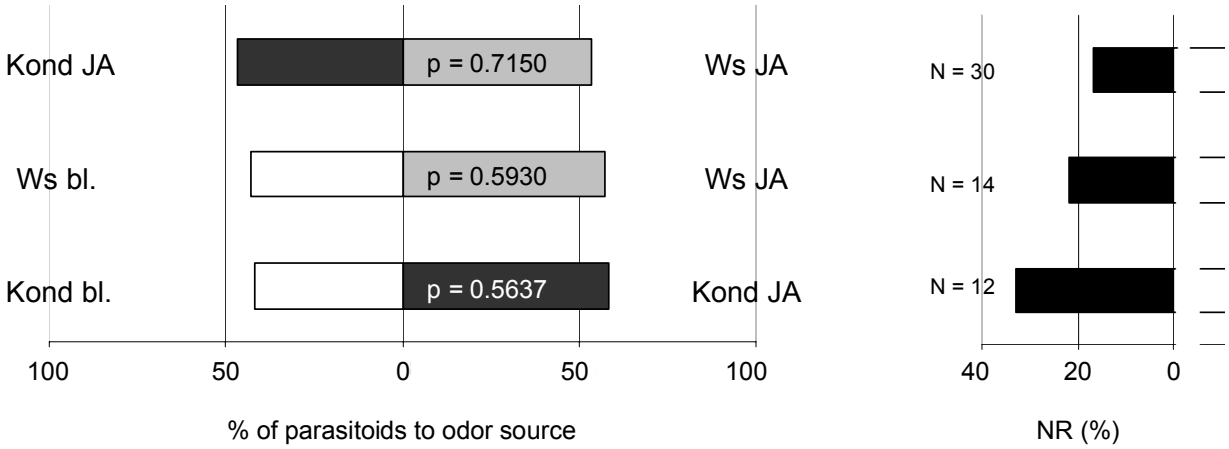

Figure 8: Response of *D. semiclausum* to Arabidopsis plants of ecotype Kond and Ws sprayed with JA or 0.1% Tween solution (bl.) in a Y-tube olfactometer bioassay. N is the number of wasps tested, NR is the number of wasps that were not responding. Choices between odor sources were analyzed with a two-sided binomial test, p-values are indicated in bars.

Results are gathered over three experimental days. Figure 8 shows that neither Kond nor Ws are significantly more attractive to *D. semiclausum* when sprayed with JA compared to the control treatment. Volatiles emitted by both Kond and Ws after induction by JA do not enable *D. semiclausum* to discriminate between JA and control plants. None of the two ecotypes is significantly more attractive when the JA-treatments are tested against each other. The volatiles emitted by Kond and Ws after induction by JA are not relevant for *D. semiclausum* to discriminate between the two ecotypes.

The average fresh weight of the leaves is in grams for Kond: JA 0.63 and bl 0.58, for Ws: JA 0.57 and bl 0.29.

Experiment 7: Kondara vs. Columbia

Do ecotypes Kond and Col0 have a natural variation in volatile production that is relevant for parasitic wasps to discriminate between these ecotypes?

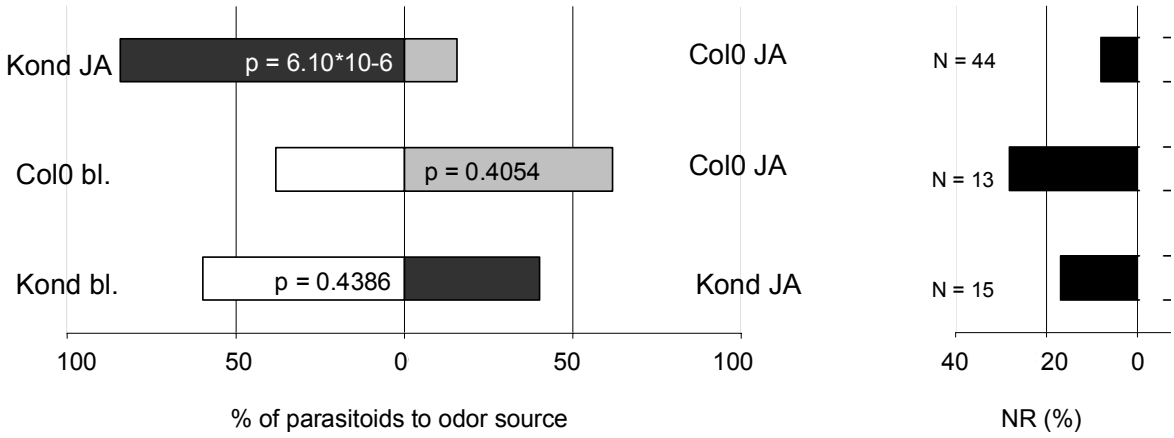

Figure 9: Response of *D. semiclausum* to Arabidopsis plants of ecotype Kond and Col0 sprayed with JA or 0.1% Tween solution (bl.) in a Y-tube olfactometer bioassay. N is the number of wasps tested, NR is the number of wasps that were not responding. Choices between odor sources were analyzed with a two-sided binomial test, p-values are indicated in bars.

Results are gathered over four experimental days. Figure 9 shows that neither Kond nor Col0 are significantly more attractive to *D. semiclausum* when sprayed with JA compared to the control treatment. Volatiles emitted by both Kond and Col0 after induction by JA do not enable *D. semiclausum* to discriminate between JA and control plants. Kond is significantly more attractive when the JA-treatments are tested against each other. So volatiles emitted by Kond and Col0 after induction by JA vary in such a way that *D. semiclausum* is able to make a discrimination between the two ecotypes.

The average fresh weight of the leaves is in grams for Kond: JA 0.99 and bl 0.77, for Col0: JA 0.91 and bl 0.71

Experiment 8: Eriengsboda vs. Columbia

Do ecotypes Eri and Col0 have a natural variation in volatile production that is relevant for parasitic wasps to discriminate between these ecotypes?

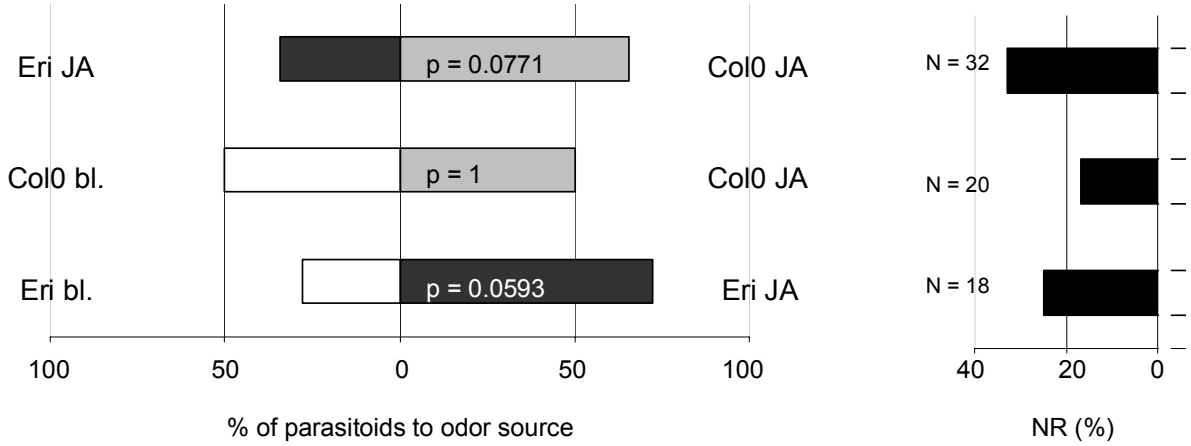

Figure 10: Response of *D. semiclausum* to Arabidopsis plants of ecotype Eri and Col0 sprayed with JA or 0.1% Tween solution (bl.) in a Y-tube olfactometer bioassay. N is the number of wasps tested, NR is the number of wasps that were not responding. Choices between odor sources were analyzed with a two-sided binomial test, p-values are indicated in bars.

Results are gathered over four experimental days. Figure 10 shows that neither Eri nor Col0 are significantly more attractive to *D. semiclausum* when sprayed with JA compared to the control treatment. Volatiles emitted by both Kond and Ws after induction by JA do not enable *D. semiclausum* to discriminate between JA and control plants. None of the two ecotypes is significantly more attractive when the JA-treatments are tested against each other. The volatiles emitted by Eri and Col0 after induction by JA are not relevant for *D. semiclausum* to discriminate between the two ecotypes.

The average fresh weight of the leaves are in grams for Eri JA: 1.11, for Col0 JA: 1.00

Experiment 9: Cape Verde Islands vs. Columbia.

Do ecotypes Cvi and Col0 have a natural variation in volatile production that is relevant for parasitic wasps to discriminate between these ecotypes?

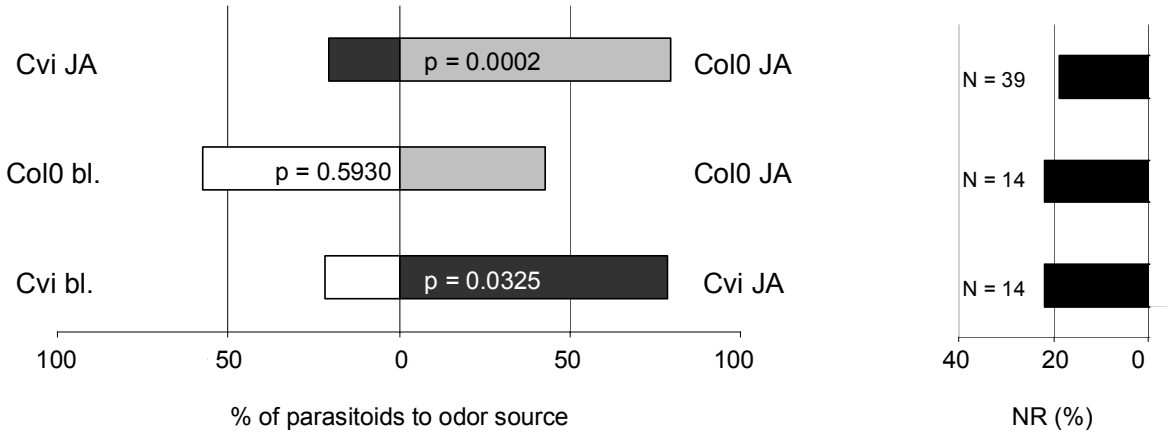

Figure 11: Response of *D. semiclausum* to Arabidopsis plants of ecotype Cvi and Col0 sprayed with JA or 0.1% Tween solution (bl.) in a Y-tube olfactometer bioassay. N is the number of wasps tested, NR is the number of wasps that were not responding. Choices between odor sources were analyzed with a two-sided binomial test, p-values are indicated in bars.

Results are gathered over four experimental days. Figure 11 shows that only Cvi is significantly more attractive to *D. semiclausum* when sprayed with JA compared to the control treatment. Volatiles emitted by Cvi after induction by JA enable *D. semiclausum* to discriminate between JA and control plants. Col0 is significantly more attractive when the JA-treatments are tested against each other. So volatiles emitted by Kond and Col0 after induction by JA vary in such a way that *D. semiclausum* is able to make a discrimination between the two ecotypes.

The average fresh weight of the leaves is in grams for Cvi: JA 0.98 and bl 1.00, for Col0: JA 1.10 and bl 1.17.

Experiment 10: Landsberg erecta vs. Columbia.

Do ecotypes Ler and Col0 have a natural variation in volatile production that is relevant for parasitic wasps to discriminate between these ecotypes?

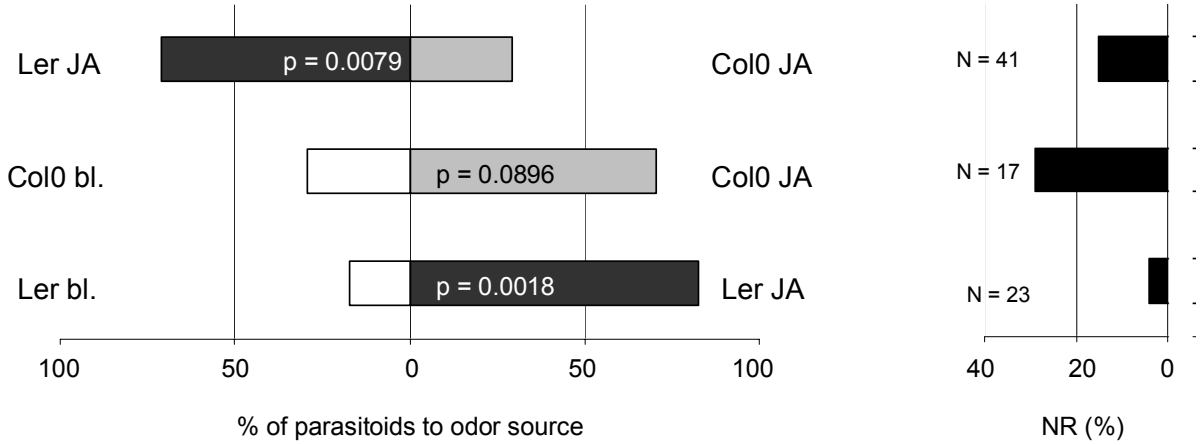

Figure 12: Response of *D. semiclausum* to Arabidopsis plants of ecotype Ler and Col0 sprayed with JA or 0.1% Tween solution (bl.) in a Y-tube olfactometer bioassay. N is the number of wasps tested, NR is the number of wasps that were not responding. Choices between odor sources were analyzed with a two-sided binomial test, p-values are indicated in bars.

Results are gathered over four experimental days. Figure 12 shows that only Ler is significantly more attractive to *D. semiclausum* when sprayed with JA compared to the control treatment. Volatiles emitted by Ler after induction by JA enable *D. semiclausum* to discriminate between JA and control plants. Ler is significantly more attractive when the JA-treatments are tested against each other. So volatiles emitted by Ler and Col0 after induction by JA vary in such a way that *D. semiclausum* is able to make a discrimination between the two ecotypes.

The average fresh weight of the leaves is in grams for Ler: JA 0.64 and bl 0.61, for Col0: JA 0.69 and bl 0.64.

Experiment 11: Wassilewskija vs. Columbia.

Do ecotypes Ws and Col0 have a natural variation in volatile production that is relevant for parasitic wasps to discriminate between these ecotypes?

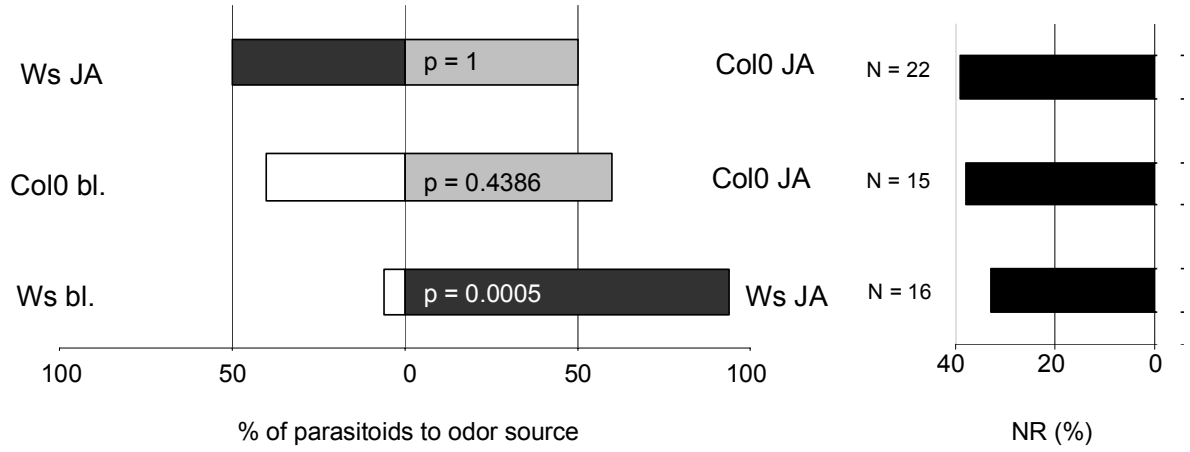

Figure 13: Response of *D. semiclausum* to Arabidopsis plants of ecotype Ws and Col0 sprayed with JA or 0.1% Tween solution (bl.) in a Y-tube olfactometer bioassay. N is the number of wasps tested, NR is the number of wasps that were not responding. Choices between odor sources were analyzed with a two-sided binomial test, p-values are indicated in bars.

Results are gathered over three experimental days. Figure 13 shows that only Ws is significantly more attractive to *D. semiclausum* when sprayed with JA compared to the control treatment. Volatiles emitted by Ws after induction by JA enable *D. semiclausum* to discriminate between JA and control plants. None of the two ecotypes is significantly more attractive when the JA-treatments are tested against each other. The volatiles emitted by Ws and Col0 after induction by JA are not relevant for *D. semiclausum* to discriminate between the two ecotypes.

The average fresh weight of the leaves is in grams for Ws: JA 0.95 and bl 1.07, for Col0: JA 0.83 and bl 0.86.

3.2 JA-treatment per ecotype

All separate experimental days in which an ecotype is involved are added to gain a better overview of JA-treatments being more attractive compared to a blanc-treatment. The reason to do this is that a larger number of tested individuals gives a better overview of the overall preference of *D. semiclausum*. JA stands for the particular ecotype sprayed with 1 mM JA + 0.1% Tween solution, bl. is the abbreviation for blank and indicates the treatment of the particular ecotype with 0.1% Tween solution (also referred to as control treatment). N is the number of parasitic wasps that responded during the experiment, while NR is the number of parasitic wasps not responding. As a statistical tool a two-sided binomial test was used, $P \geq 0.05$ is not significant; $P < 0.05$ is significant.

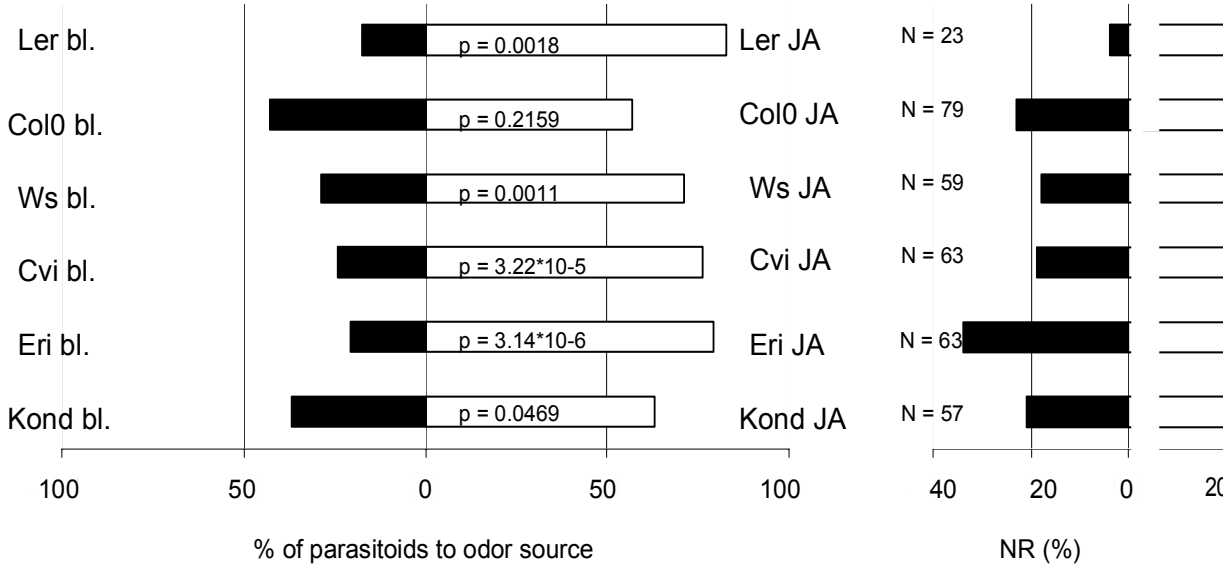

Figure 14: : Response of *D. semiclausum* to Arabidopsis plants per ecotypes, sprayed with 1,0 mM JA + 0.1% Tween or 0.1% Tween (bl.) in a Y-tube olfactometer bioassay. N is the number of wasps tested, NR is the number of wasps that were not responding.

All ecotypes except Col0 are significantly more attractive to *D. semiclausum* when sprayed with JA compared to the control plants.

4. Discussion

Plants are constantly under attack by herbivorous insects. Direct defense forms a mechanism involving only two trophic levels, the plant and the herbivore. Indirect defense involves the participation of a third trophic level, a predator or parasitoid of the herbivore (Vet and Dicke, 1992). After a plant is damaged by a feeding herbivore it starts to emit *de novo* produced volatiles, so called herbivore induced plant volatiles (HIPVs). These volatiles are intercepted by predators or parasitoids and help them to locate the herbivore feeding on the plant (Van Poecke and Dicke, 2004). A difference can be observed between the quantity and quality of emitted HIPVs between plant species and even between plant cultivars and ecotypes (Krips *et al*., 2001). As *A. thaliana* is a plant species with a large dispersal area there exist numerous ecotypes each having a complex genetic background (Raven *et al*., 2003). This genetic variation between ecotypes might be an indication of differences in HIPV blends of ecotypes (Gouinguené *et al*., 2001). In this thesis I gained insight in attractiveness of HIPV blends emitted by six Arabidopsis ecotypes by conducting two-choice behavioral assays. For this, parasitoid *D. semiclausum* was used to discriminate amongst different HIPV ecotypes.

To place the gathered results in a larger picture, a short overview of Arabidopsis volatiles in literature will be provided.
Kuśnierczyk *et al*. compared the ecotypes Ws and Cvi and demonstrated that Cvi lowers the fitness of specialistic herbivore feeders whereas Ws did not. Although this is a form of direct defense, it might be an indication that Ws has a lower production or induction of JA (Kuśnierczyk *et al*., 2007). Infestation of Ws and Cvi, with an herbivore, caused up regulation of 60 genes in Ws and 21 in Cvi (Kuśnierczyk *et al*., 2007). When Ws and Cvi were compared we observe there is a slight preference for Ws-JA when compared to Cvi-JA (Fig. 6), this indicates that if the induction/production of JA in Ws is lower compared to Cvi, this effect is eliminated when JA is sprayed on the Ws plants.
Also Cipollini *et al*. discovered a weak direct defense in Ws as the growth rate of *Spodoptera exigua* caterpillars was higher on Ws than on Col0. This difference can largely be explained by reduced GS levels in Ws compared to Col0 (Cipollini *et al*., 2004). If Ws has a weak induction/production of JA compared to Col0 this will result in a significant attraction of *D. semiclausum* to Col0 when compared to Ws. If GSs play a large role in attracting *D. semiclausum* there will be a preference for Col0-JA when compared to Ws-JA. When Ws is compared to Col0 there is no preference for Ws-JA or Col0-JA, while Ws-JA shows to be attractive compared to Ws-bl (Fig. 13). Again Ws-JA shows to be equally attractive compared to another JA-sprayed ecotype, so if the JA induction/production in Ws is low compared to Col0, this effect is eliminated when Ws is sprayed with JA. To draw a conclusion about the importance of volatile GS degradation products it is important to know if the amount of GSs gives an accurate representation of the amount of volatile degradation products. If this is the case, volatile GS degradation products do not play a large role in attracting *D. semiclausum* as in the combination Col0 vs. Ws there is no preference for Col0 that has larger amounts of GS levels compared to Ws.
If the induction or production of JA in Ws is indeed low it can be speculated that this reduced induction/production of JA can be restored when JA is applied to the plant. When comparing the control plant to the JA treated plant a preference would be expected for the JA treated plant. If however the perception of JA or JA-products is low in Ws, application of JA will have no effect. We see an overall significant preference for JA treated Ws compared to the control untreated Ws, but not for all individual experiments. When ecotypes Ws vs. Cvi were compared, no preference for Ws-JA over Ws-bl was observed (Fig. 6). So even though it does not show in all the experiments, if it is true that Ws showed a lower production or induction

of JA this is restored by spraying JA. To investigate if Ws has a lower induction/production of JA, no JA should be sprayed on the plant to ensure all JA present is produced by the plant itself. A controlled rate of herbivory or artificial damage can be inflicted to Ws and the other ecotype to investigate how this affects the induction/production of JA and consequently the emission of HIPVs by both ecotypes.

Ecotype Cvi emits no sesquiterpenes, an important group of floral volatiles (Tholl *et al*., 2005), but shows a strong induction of the JA signal transduction pathway compared to ecotype Ws (Kuśnierczyk *et al*., 2007). If sesquiterpenes are an important group of volatiles that attract *D. semiclausum* it is expected that Cvi is not attractive when compared to other sesquiterpene emitting ecotypes. When combining Cvi and Col0, Cvi-JA is significantly less attractive than Col0-JA (Fig. 11). If Cvi-JA is compared to JA-treatments of Kond, Ws and Eri, Cvi-JA is slightly less attractive than the JA-treatment of the other ecotypes (Fig. 4, 6 and 7). This suggests that sesquiterpenes play a role in attraction of *D. semiclausum* but contribute only partially in creating an attractive Arabidopsis volatile blend.

A preliminary headspace analysis of Kond and Eri revealed that there are some notable differences when quantities of some volatile compounds are compared (Snoeren, pers. com.). For 15 compounds is demonstrated an at least two-fold more abundance for Kond versus Eri emitting plants. In contrast there are no compounds at least two-fold more abundant in the Eri volatile blend. The most abundant compounds (24-37 times more abundant in Kond) are terpenoids including monoterpenes (E)- ß-ocimene (which can lead to parasitoid attraction (Fäldt *et al*, 2002)), α-phellandrene (Jost *et al*., 2008) and α-terpineol, and sesquiterpenes ß-caryophyllene (Agrawal *et al*. 1999), ß-chamigrene and ß-sesquiphellandrene (Tholl *et al*. 2005). According to this information Kond will be more attractive than Eri if terpenoids attract *D. semiclausum.* This hypothesis seems right as the results show that Kond-JA is significantly more attractive when compared to Eri-JA (Fig. 3). Eri-JA is not completely unattractive as it is still significantly more attractive when compared to Eri-bl. When Kond is compared to Ws we observe that *D. semiclausum* has no preference when Kond-JA is compared to Ws-JA (Fig. 8). Ws-JA seems equally attractive as Kond-JA, from this we would expect that Ws-JA is more attractive than Eri-JA, however this is not the case (Fig. 5). The same holds true for Cvi, as Kond-JA is equally attractive as Cvi-JA (Fig. 4), we would expect Cvi-JA to be more attractive than Eri-JA. Again, this is not the case as Eri-JA is even slightly more attractive than Cvi-JA (Fig. 7). These results are not logically explainable according to the data.

Col0 contains a deletion in the *CYP74B2*-gene which codes for an incomplete hydroperoxide lyase (HPL) protein which is involved in the production of GLVs. In the leaves of the Col0 ecotype certain transcripts are expressed at significantly lower amounts compared to other ecotypes; this is a possible effect of the difference in HPL expression. These transcripts are involved in the production of C6-volatiles (green leaf volatiles), jasmonates and glucosinolates, all involved in induced direct or indirect defense (Duan *et al*., 2005). As ecotype Col0 emits very low amounts of GVLs, it is to be expected that attraction of parasitoids will not be so successful. The results reveal a different view as in some experiments Col0 is surprisingly attractive to *D. semiclausum* when compared to other ecotypes. When compared to Cvi-JA, Col0-JA is significantly more attractive (Fig. 11) and compared to Eri-JA, Col0-JA is slightly more attractive (Fig. 10). When we compare Kond-JA and Col0-JA we see a significant preference for Kond-JA (Fig. 9). Kond-JA emits large quantities of terpenoids compared to Eri-JA (Snoeren, pers. com.), compared to Kond-JA, Col0-JA is less attractive, compared to Eri-JA, Col0-JA is slightly more attractive. Taking

these results into consideration I expect the quantity of terpenoids emitted by Col0-JA to be in between the quantities emitted by Kond-JA and Eri-JA. Kond-JA is significantly more attractive than Col0-JA, Col0-JA is however significantly more attractive than Cvi-JA. Comparing these results it might be expected that the combination Kond-JA vs. Cvi-JA will give a significant preference for Kond-JA, this is however not the case as *D. semiclausum* has no preference for Kond-JA over Cvi-JA (Fig. 4).

Compared to Ler, Col0 has a lower expression level of transcripts involving LOX2, LOX3, GSs and JAs like OPDA reductase (OPR3) (Duan *et al.*, 2005). JAs are one of the most important classes of signaling chemicals after wounding or herbivore-feeding and are involved in the induction of HIPVs (Halitschke and Baldwin, 2005). Compared to Ler, leaves of Col0 plants contained a reduced amount of hexanal and no trans-2-hexanal (Duan *et al.*, 2005). An Arabidopsis mutant with an impairment somewhere in the LOX/lyase pathway emits compared to the wild type plant less (Z)-3-hexanal, a GLV. *Cotesia glomerata* was less attracted to this mutant after herbivore damage compared to the wild type plant (Shiojiri *et al.*, 2006). As Col0 has a reduced level of several very important signaling chemicals and attractive HIPV compounds compared to Ler, I would expect a preference for Ler-JA when compared to Col0-JA. The results confirm this as there is a significant preference for Ler-JA when compared to Col0-JA (Fig. 12).
This was the only combination made with Ler so I can only speculate what the results will be if the other ecotypes are also combined with Ler. The combination Kond-JA vs. Ler-JA will be interesting as both ecotypes showed to be significantly more attractive than Col0-JA. As there is nothing known about the volatile blend profile when Ler-JA is compared to Kond-JA it is difficult to predict which ecotype will be more attractive. Kuśnierczyk *et al*. demonstrated that Cvi and Ler have a somewhat different herbivore-defense strategy. Cvi has a strong induction of the JA pathway while in Ler the indole glucosinolate synthesis pathway was induced. When Cvi, Ws and Ler were compared, Cvi had the strongest induction of the JA-synthesis pathway after herbivore infestation (Kuśnierczyk *et al*. 2007). We observed an equally attractiveness for Col0-JA and Ws-JA, now it is tempting to say Ler-JA will be more attractive than Ws-JA. However we need to take into consideration that all combinations made with Ws showed that Ws-JA is equally or even slightly more attractive than the other ecotypes. So the combination Ler-JA vs. Ws-JA might also be an interesting one. When looking at the results, Ler-JA will be significantly more attractive than Cvi-JA as Col0-JA already showed to be more attractive than Cvi-JA. However, the results from Kuśnierczyk *et al*. show a stronger induction of the JA-synthesis pathway for Cvi compared to Ler. These results however are gathered after infestation with an herbivore, probably JA-spraying will give another result. When Ler-JA is compared to Eri-JA I expect a significant preference for Ler-JA as Col-JA is slightly more attractive to *D. semiclausum* when compared to Eri-JA.

All ecotypes (Ws, Ler, Kond, Eri and Cvi) except Col0 are more attractive to *D. semiclausum* when sprayed with JA compared to the control treatment. Experiments done earlier by Van Poecke and Dicke indicated that spraying of Arabidopsis with JA increased the attraction to specific parasitoids (Van Poecke and Dicke, 2002). Spraying with JA induces the emission of a volatile blend that enables *D. semiclausum* to discriminate between JA- and control treatment. Spraying ecotype Col0 with a 1 mM JA-solution increased the attractiveness significantly for both *C. rubecula* and *D. semiclausum* when compared to untreated control plants (Van Poecke and Dicke, 2002; Snoeren, unpublished data). I propose that JA-treated Col0 emits volatiles repellant to *D. semiclausum.* However, Col0-JA plants have proven to be attractive when compared to other JA-treated ecotypes. Rohloff and Bones discovered that undamaged Arabidopsis leaves and flowers emit a blend of 24 mono- and 26 sesquiterpenes.

The ecotypes they compared (Col0, Cvi, Ler and Ws) showed no differences in monoterpene profiles (Rohloff and Bones, 2005). The only plausible explanation is that control plants of Col0 already emit a considerable constitutive amount of volatiles that attract *D. semiclausum* wasps, possibly these are mono- or sesquiterpenes.

If all the results are taken into consideration a rough conclusion might be drawn about the importance of different HIPV compounds that attract *D. semiclausum.* GSs do not seem very important in attraction of *D. semiclausum* as Ws has a reduced amount of GSs compared to Col0 (Cipollini *et al*., 2004), but the two are equally attractive (Fig. 13). GLVs do also not seem to be very attractive to *D. semiclausum* as Col0 emits no GLVs (Duan *et al*., 2005) but is in certain combinations very attractive (Fig. 10 and 11). Mumm *et al*. discovered that GLVs are also not playing a big role in attraction of parasitoid *C. glomerata*. Brussels sprouts treated with fosmidomycin (terpenoid inhibitor) showed a reduced emission of GLVs after infestation with *P. brassicae* compared to infested control (water treated) plants. *C. glomerata* did not discriminate between infested fosmidomycin treated plants and infested control plants. This might be an indication that GLVs are not used by *C. glomerata* to locate a host infested plant (Mumm *et al*., 2008). Terpenoids seem to be a very important groups of HIPVs to attract *D. semiclausum*. Cvi emits no sesquiterpenes (Tholl *et al*., 2005) and is significantly less attractive than Col0 (Fig. 11) and slightly less attractive compared to other ecotypes (Fig. 4, 6 and 7). Also combination Kond vs. Eri confirm a strong preference of *D. semiclausum* as Kond-JA emits larger quantities of certain terpenoids compared to Eri-JA (Snoeren, pers. com.). *D. semiclausum* significantly prefers Kond over Eri (Fig. 3). Mumm *et al*. showed that lima bean infested by a spider mite and treated with fosmidomycin emits lower amounts or even completely inhibits the emission of several sesqui-, homo- and monoterpenes compared to infested control plants. Infested fosmidomycin lima bean plants are less attractive to a predatory mite compared to infested control plants. Brussels sprouts infested with *P. brassicae* and treated with fosmidomycin emitted no significantly lower amount of monoterpenes compared to infested control plants. *C. glomerata* also could not discriminate between infested fosmidomycin and infested control plants (Mumm *et al*., 2008). All these results point towards an important role of terpenoids in the attraction of predators and parasitoids.

In nature *D. semiclausum* is not interacting with Arabidopsis as their host *Plutella xylostella* is not a natural herbivore of this plant. It is an interesting observation that the tritrophic system works despite that it is not evolved in nature. This indicates that volatile emission by Arabidopsis is not specifically targeted to specialist parasitoids of Arabidopsis pest. *D. semiclausum* does not discriminate between Col0 plants infested with its host *P.xylostella* and non-host *P. rapae* (Snoeren, unpublished data). The herbivore-specific recognition in Arabidopsis or the difference between the HIPV blend emitted after host or non-host infestation is not sufficient for *D. semiclausum* to make a discrimination.

A remarkable thing to see is that for some of the other ecotypes also no significant preference for JA-treatment is observed in the separate experiments. In some cases the results are very close to significance. However, when all experiments are combined there is an overall preference for JA-treatment above control treatment. This effect may be explained by the number of wasps tested. If this number is small as in the separate experiments it is more difficult to reach significance, as higher number give a better overall indication of preferences.

Gouinguené *et al*. noticed that in maize cultivars there can be a difference in time between plant damage and HIPV emission and the peaks in volatile emission between the different genotypes. It is not impossible that a similar system exists in Arabidopsis ecotypes. This might mean that for certain ecotypes the time an experiment is conducted falls outside the volatile emission peak which makes the ecotype less attractive at that time (Gouinguené *et al*. 2001). As one experimental day takes several hours the data might show a fluctuation in attractiveness of a certain ecotype during the experiment.

Researchers are still working hard to get a better insight in the variation in HIPV emission and the underlying natural genetic variation. As we have seen in this thesis report, there indeed is a difference in volatile emission that enables parasitic wasp *D. semiclausum* to discriminate between ecotypes. Next to the genetic differences causing the diversity in HIPV blend there may be numerous other genetic polymorphisms causing phenotypic diversity amongst Arabidopsis ecotypes.

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Appendix

Combination Kond vs. Cvi We 7/11/07

Wasp	First choice	Time	Switches	Final choice	Time
Kond					
1	JA		-	JA	0.25
2	JA		1	Blanc	4.14
3	JA		-	JA	2.47
4	JA		-	JA	8.41
5	Blanc	4.44	2	Blanc	9.28
6	NR	*	*	*	*
Cvi					
7	JA	1.19	-	JA	1.28
8	JA	0.24	4	JA	5.02
9	JA	0.27	-	JA	3.34
10	NR	*	*	*	*
11	JA	1.01	-	JA	1.12
12	Blanc	1.07	-	Blanc	2.58
Kond JA	**vs. Cvi JA**				
13	Kond	2.45	-	Kond	3.17
14	Cvi	2.24	1	Kond	3.57
15	NR	*	*	*	*
16	NR	*	*	*	*
17	Kond	0.22	4	Kond	7.25
18	Cvi	3.25	-	Cvi	4.03
19	Kond	1.03	2	*	*
20	Cvi	0.15	-	Cvi	0.31
21	Cvi	4.45	1	Kond	5.28
22	Cvi	0.43	-	Cvi	1.09
23	Kond	0.10	-	Kond	0.19
24	Kond	0.47	1	Cvi	3.23

Weight: Cvi JA: 0.73/0.32/0.72/0.44
Kond blanc: 0.74/0.78/0.49/0.82
Cvi Blanc: 0.40/1.14/0.63/0.41
Kond JA: 0.71/0.61/0.68/0.70

Combination Eri vs. Ws Th 8/11/07

Wasp	First choice	Time	Switches	Final choice	Time
Eri					
1	NR	*	*	*	*
2	Blanc	0.27	4	Blanc	3.21
3	Blanc	0.10	-	Blanc	3.00
4	JA	0.11	-	JA	0.32
5	NR	*	*	*	*
6	blanc	3.25	3	JA	4.56
Ws					
7	JA	0.18	-	JA	0.42
8	NR	*	*	*	*
9	JA	2.08	1	blanc	2.57
10	Blanc	3.07	1	JA	9.52
11	JA	2.07	-	JA	5.37
12	JA	1.47	4	JA	6.26
Eri JA	**Vs. Ws JA**				
13	Ws	1.31	-	Ws	2.31
14	Ws	0.29	-	Ws	0.57
15	Ws	1.06	-	Ws	1.42
16	Ws	4.11	-	Ws	4.45
17	Eri	0.15	1	Ws	1.42
18	Ws	1.34	2	Ws	6.26
19	Ws	0.21	-	Ws	0.51
20	Ws	0.47	-	Ws	1.16
21	NR	*	*	*	*
22	Eri	2.36	-	Eri	6.17
23	Ws	1.40	-	Ws	2.08
24	Ws	1.19	1	Eri	2.47

Weight: Eri blanc: 0.59/0.71/0.53/0.80
Eri JA: 0.59/0.70/0.58/0.48
Ws blanc: 1.02/0.66/0.83/0.83
Ws JA: 0.45/0.83/0.93/1.05

Combination Kond vs. Eri We 14/11/07

Wasp	First choice	Time	Switches	Final choice	Time
Kond					
1	JA	0.31	-	JA	1.08
2	Blanc	0.13	1	JA	0.51
3	JA	0.09	-	JA	0.56
4	JA	0.48	-	JA	1.10
5	JA	0.11	-	JA	0.36
6	JA	0.16	-	JA	0.40
Eri					
7	Blanc	0.10	1	JA	2.48
8	JA	0.27	-	JA	1.00
9	JA	0.32	-	JA	0.56
10	NR	*	*	*	*
11	JA	0.10	-	JA	0.27
12	JA	0.45	-	JA	1.47
Kond JA	**Vs. Eri JA**				
13	Kond	2.10	-	Kond	3.28
14	Eri	0.12	-	Eri	1.19
15	Kond	0.31	-	Kond	1.00
16	Kond	0.09	-	Kond	0.31
17	Eri	0.33	1	Kond	0.57
18	Kond	0.08	-	Kond	0.33
19	Kond	4.15	-	Kond	6.44
20	Eri	1.12	-	Eri	1.35
21	Kond	0.12	-	Kond	0.30
22	Eri	0.12	1	Kond	0.47
23	Kond	0.23	2	Kond	2.13
24	Kond	2.06	-	Kond	2.58

Weight: Kond blanc: 1.17/1.03/1.33/0.74
Eri blanc: 0.45/0.83/0.52/0.78
Kond JA: 1.49/1.39/0.71/0.55
Eri JA: 0.82/0.88/0.61/0.65

Combination Cvi vs. Ws Tu 20/11/07

Wasp	First choice	Time	Switches	Final choice	Time
Cvi					
1	Blanc	0.08	-	*	*
2	Blanc	1.25	1	JA	2.54
3	JA	1.13	6	JA	7.04
4	Blanc	1.12	-	Blanc	2.04
5	Blanc	0.28	1	JA	4.03
6	Blanc	1.37	-	Blanc	2.45
Ws					
7	NR	*	*	*	*
8	NR	*	*	*	*
9	JA	4.43	-	JA	5.06
10	JA	0.08	3	Blanc	4.18
11	Blanc	0.11	-	Blanc	0.42
12	Blanc	2.19	-	Blanc	2.59
Cvi JA	**Vs. Ws JA**				
13	Cvi	1.06	-	Cvi	1.53
14	Cvi	0.31	-	Cvi	4.00
15	Cvi	1.12	-	Cvi	2.13
16	Ws	0.15	1	Cvi	1.44
17	Ws	0.14	-	Ws	0.34
18	Cvi	0.10	-	Cvi	0.32
19	Cvi	3.21	-	Cvi	3.42
20	NR	*	*	*	*
21	Ws	1.04	-	Ws	2.37
22	Ws	1.40	-	Ws	2.16
23	Ws	0.15	-	Ws	1.12
24	Ws	2.33	-	Ws	4.03

Weight: Cvi blanc: 0.30/0.26/0.27/0.32
Ws blanc: 0.26/0.25/0.24/0.25
Cvi JA: 0.28/0.24/0.26/0.25
Ws JA: 0.15/0.23/0.37/0.30

Combination Kond vs. Eri We 21/11/07

Wasp	First choice	Time	Switches	Final choice	Time
Kond					
1	NR	*	*	*	*
2	Blanc	4.01	3	JA	7.27
3	NR	*	*	*	*
4	JA	1.00	2	JA	2.00
5	JA	0.05	-	JA	1.15
6	JA	1.11	-	JA	1.50
Eri					
7	JA	1.55	2	JA	5.35
8	NR	*	*	*	*
9	JA	0.20	-	JA	0.40
10	JA	1.25	3	Blanc	6.01
11	NR	*	*	*	*
12	NR	*	*	*	*
Kond JA	**Vs. Eri JA**				
13	Kond	1.32	3	Kond	5.02
14	NR	*	*	*	*
15	Eri	0.19	1	Kond	1.31
16	Kond	0.25	-	Kond	0.44
17	Kond	3.37	2	Kond	4.37
18	Eri	3.23	1	*	*
19	Kond	1.35	-	Kond	3.08
20	NR	*	*	*	*
21	Kond	4.59	-	Kond	8.19
22	Kond	0.09	-	Kond	0.30
23	Eri	0.23	1	Kond	2.45
24	Eri	1.25	-	Eri	1.53

Weight: Kond blanc: 0.35/0.38/0.34/0.32
Eri blanc: 0.23/0.25/0.29/0.35
Eri JA: 0.30/0.27/0.24/0.26
Kond JA: 0.35/0.38/0.31/0.34

Combination Kond vs. Ws Th 22/11/07

Wasp	First choice	Time	Switches	Final choice	Time
Ws					
1	Blanc	0.36	2	Blanc	2.52
2	Blanc	0.19	2	*	*
3	JA	0.33	3	*	*
4	Blanc	1.34	1	JA	4.17
5	NR	*	*	*	*
6	JA	0.06	1	blanc	3.56
Kond					
7	NR	*	*	*	*
8	JA	1.36	*	*	*
9	JA	4.45	*	*	*
10	JA	0.59	1	Blanc	1.52
11	JA	0.10	-	JA	0.31
12	NR	*	*	*	*
Kond JA	**Vs. Ws JA**				
13	Ws	0.58	-	Ws	1.21
14	Ws	2.04	-	Ws	2.48
15	Ws	1.55	-	Ws	4.10
16	NR	*	*	*	*
17	Ws	0.17	1	Kond	1.11
18	Kond	1.08	-	Kond	1.28
19	Ws	2.00	-	Ws	3.07
20	Kond	3.25	1	*	*
21	Ws	0.44	-	Ws	1.21
22	Ws	2.26	1	Kond	3.30
23	Ws	2.38	-	Ws	3.55
24	Kond	0.49	6	Kond	6.14

Weight: Ws Blanc: 0.40/0.24/0.32/0.20
Kond Blanc: 0.27/0.29/0.33/0.30
Ws JA: 0.19/0.33/0.35/0.27
Kond JA: 0.32/0.29/0.42/0.32

Combination Kond vs. Cvi Tu 27/11/07

Wasp	First choice	Time	Switches	Final choice	Time
Kond					
1	Blanc	0.11	1	JA	1.54
2	Blanc	3.48	-	Blanc	5.00
3	JA	3.28	*	*	*
4	NR	*	*	*	*
5	JA	0.14	-	JA	2.13
6	Blanc	0.15	1	JA	1.17
7	JA	0.20	-	JA	0.47
8	JA	0.11	-	JA	1.10
Cvi					
9	Blanc	0.14	1	JA	2.33
10	JA	0.35	-	JA	0.53
11	JA	2.01	-	JA	2.23
12	JA	1.02	5	*	*
13	JA	0.35	2	JA	2.13
14	JA	0.24	-	JA	1.02
15	NR	*	*	*	*
16	JA	0.11	-	JA	1.03
Kond JA	**Vs. Cvi JA**				
17	Kond	0.14	-	Kond	0.36
18	Kond	4.55	2	Kond	8.01
19	Kond	0.13	-	Kond	0.48
20	Kond	2.44	-	Kond	3.13
21	Cvi	0.10	-	Cvi	0.30
22	Cvi	0.09	-	Cvi	0.32
23	Kond	0.31	-	Kond	1.59
24	Kond	0.29	2	Kond	1.50
25	Cvi	0.25	4	Cvi	7.36
26	NR	*	*	*	*
27	NR	*	*	*	*
28	Kond	1.02	2	Kond	2.26

Weight: Kond Blanc: 0.50/0.38/0.39/0.47
Cvi Blanc: 0.57/0.36/0.57/0.62
Kond JA: 0.45/0.50/0.63/0.44
Cvi JA: 0.63/0.61/0.71/0.63

Combination Eri vs. Cvi Tu 4/12/07

Wasp	First choice	Time	Switches	Final choice	Time
Eri					
1	NR	*	*	*	*
2	NR	*	*	*	*
3	NR	*	*	*	*
4	Blanc	0.09	3	JA	3.45
5	JA	0.08	-	JA	0.30
6	NR	*	*	*	*
Cvi					
7	Blanc	0.05	3	JA	6.44
8	JA	0.18	-	JA	1.02
9	NR	*	*	*	*
10	JA	1.45	-	JA	4.22
11	Blanc	3.00	-	Blanc	4.00
12	NR	*	*	*	*
Eri JA	**Vs. Cvi JA**				
13	Cvi	0.25	-	*	*
14	NR	*	*	*	*
15	Cvi	0.10	-	Cvi	4.16
16	Cvi	0.30	1	Eri	1.55
17	NR	*	*	*	*
18	Cvi	3.20	-	Cvi	4.42
19	NR	*	*	*	*
20	Cvi	2.48	-	Cvi	3.55
21	Cvi	0.12	-	Cvi	0.33
22	Cvi	1.19	-	Cvi	2.51
23	Eri	0.10	1	Cvi	1.21
24	NR	*	*	*	*

Weight: Eri Blanc: 0.65/0.62/0.46/0.59
Cvi Blanc: 0.51/0.27/0.33/0.36
Cvi JA: 0.39/0.27/0.43/0.39
Eri JA: 0.53/0.62/0.68/0.69

Combination Cvi vs. Ws We 5/12/07

Wasp	First choice	Time	Switches	Final choice	Time
Cvi					
1	JA	0.08	-	JA	0.30
2	Blanc	0.56	2	Blanc	5.38
3	JA	2.49	1	Blanc	3.23
4	JA	0.23	-	JA	2.45
5	NR	*	*	*	*
6	JA	1.41	-	JA	3.36
Ws					
7	Blanc	2.32	-	Blanc	4.29
8	Blanc	1.24	-	Blanc	2.37
9	Blanc	1.49	*	*	*
10	JA	0.07	-	JA	2.45
11	JA	0.15	-	JA	2.01
12	JA	1.01	-	JA	1.57
Cvi JA	**Vs. Ws JA**				
13	Ws	0.18	-	Ws	2.08
14	Ws	1.19	-	Ws	3.03
15	Ws	0.05	-	Ws	0.51
16	NR	*	*	*	*
17	Ws	3.12	-	Ws	4.00
18	Ws	2.26	-	Ws	3.13
19	Ws	1.00	-	Ws	3.09
20	Cvi	1.11	2	Cvi	6.10
21	Ws	1.59	-	Ws	2.28
22	Cvi	0.09	1	Ws	1.10
23	Cvi	1.07	-	Cvi	1.43
24	Ws	4.28	1	*	*

Weight: Cvi Blanc: 0.42/0.43/0.31/0.54
Ws Blanc: 0.32/0.49/0.36/0.31
Cvi JA: 0.38/0.41/0.34/0.49
Ws JA: 0.32/0.38/0.46/0.35

Remark: Ws plants sprayed with JA show purple coloration.

Combination Kond vs. Eri Th 6/12/07

Wasp	First choice	Time	Switches	Final choice	Time
Eri					
1	JA	1.45	-	JA	2.20
2	Blanc	1.19	1	JA	2.25
3	JA	0.15	-	JA	4.28
4	JA	1.48	2	JA	5.58
5	JA	2.52	-	JA	4.55
6	JA	2.39	1	Blanc	4.55
Kond					
7	Blanc	1.31	*	*	*
8	Blanc	1.47	-	Blanc	2.06
9	JA	3.23	1	Blanc	4.13
10	Blanc	0.12	-	Blanc	0.33
11	Blanc	0.14	1	JA	2.20
12	JA	0.13	-	JA	1.21
Eri JA	**Vs.Kond JA**				
13	Kond	3.18	-	Kond	3.46
14	Eri	0.09	1	Kond	1.12
15	NR	*	*	*	*
16	Kond	0.12	*	*	*
17	Kond	0.23	-	Kond	0.45
18	Kond	1.22	1	Eri	2.37
19	Eri	0.35	-	Eri	0.59
20	Kond	1.09	-	Kond	1.33
21	Eri	0.08	-	Eri	0.32
22	Eri	0.33	1	Kond	1.04
23	Eri	2.05	-	Eri	2.54
24	Eri	1.15	1	Kond	2.57

Weight: Eri Blanc: 0.86/0.71/0.88/0.80
Eri JA: 0.81/0.80/0.76/0.78
Kond Blanc: 0.97/0.85/0.95/1.14
Kond JA: 0.82/0.97/0.81/0.83

Combination Kond vs. Cvi Tu 18/12/2007

Wasp	First choice	Time	Switches	Final choice	Time
Cvi					
1	JA	0.05	-	JA	0.23
2	NR	*	*	*	*
3	JA	2.40	-	JA	4.55
4	JA	2.33	-	JA	3.14
5	JA	0.20	2	JA	6.15
6	NR	*	*	*	*
Kond					
7	JA	0.11	*	*	*
8	Blanc	0.31	1	JA	3.03
9	Blanc	1.02	-	Blanc	1.21
10	NR	*	*	*	*
11	JA	0.14	-	JA	1.33
12	JA	2.37	-	JA	3.37
Cvi JA	**Vs.Kond JA**				
13	NR	*	*	*	*
14	Kond	0.08	3	*	*
15	Kond	0.34	-	Kond	1.17
16	Cvi	0.59	1	Kond	9.52
17	NR	*	*	*	*
18	Cvi	1.35	1	Kond	2.49
19	Kond	0.17	1	Cvi	2.14
20	Kond	0.10	-	Kond	1.12
21	Cvi	1.12	-	Cvi	2.42
22	Cvi	0.07	-	Cvi	0.53
23	Cvi	1.52	-	Cvi	2.31
24	Kond	3.10	1	Cvi	4.46

Weight: Cvi Blanc: 0.42/0.38/0.38/0.36
Kond Blanc: 0.38/0.52/0.73/0.64
Cvi JA: 0.37/0.48/0.45/0.46
Kond JA: 0.86/0.71/0.84/0.79

Combination Eri vs. Ws We 19/12/2007

Wasp	First choice	Time	Switches	Final choice	Time
Eri					
1	NR	*	*	*	*
2	JA	2.05	-	JA	4.39
3	JA	0.26	-	JA	3.31
4	JA	0.17	-	JA	0.38
5	JA	1.49	1	Blanc	5.16
6	JA	0.30	-	JA	0.49
Ws					
7	NR	*	*	*	*
8	JA	1.33	-	JA	6.16
9	JA	1.36	-	JA	2.32
10	JA	0.13	-	JA	0.48
11	JA	2.34	-	JA	2.52
12	Blanc	0.11	-	Blanc	0.33
eri JA	**Vs. Ws JA**				
13	Eri	0.33	4	Eri	2.35
14	NR	*	*	*	*
15	Eri	1.41	-	Eri	2.13
16	Ws	2.38	-	Ws	3.45
17	Eri	2.35	2	Eri	6.33
18	NR	*	*	*	*
19	Eri	0.09	1	*	*
20	Eri	0.15	-	Eri	1.26
21	Ws	0.15	-	Ws	0.41
22	Eri	0.23	2	Eri	6.04
23	Eri	0.46	-	Eri	1.53
24	Eri	0.17	-	Eri	2.05

Weight: Eri Blanc: 0.63/0.69/0.84/0.86
Ws Blanc: 0.59/0.53/0.64/0.55
Eri JA: 0.91/0.91/0.90/0.74
Ws JA: 0.82/1.04/0.90/0.70

Combination Cvi vs. Eri Tu 15/01/08

Wasp	First choice	Time	Switches	Final choice	Time
Cvi					
1	NR	*	*	*	*
2	NR	*	*	*	*
3	Blanc	2.00	1	JA	3.22
4	JA	0.12	-	JA	1.14
5	Blanc	0.28	-	Blanc	4.13
6	NR	*	*	*	*
Eri					
7	Blanc	0.54	3	JA	5.04
8	JA	0.09	-	JA	0.31
9	Blanc	0.40	1	JA	1.45
10	Blanc	0.37	-	Blanc	7.27
11	NR	*	*	*	*
12	Blanc	0.03	-	Blanc	0.30
Cvi JA	**Vs. Eri JA**				
13	Cvi	2.26	-	Cvi	9.13
14	Eri	0.12	-	Eri	0.31
15	Cvi	0.38	-	Cvi	4.56
16	Eri	0.16	4	Eri	4.39
17	Eri	0.03	-	Eri	0.24
18	Cvi	2.10	-	Cvi	3.01
19	Eri	0.24	1	Cvi	2.31
20	Eri	0.07	-	Eri	1.21
21	Cvi	0.19	-	Cvi	0.38
22	Cvi	2.04	*	*	*
23	Eri	0.55	-	Eri	3.40
24	Cvi	0.13	-	Cvi	1.06

Weight: Cvi JA: 0.81/0.58/0.74/0.73
Kond JA: 0.92/0.93/0.93/0.91

Combination Eri vs. Ws We 16/01/08

Wasp	First choice	Time	Switches	Final choice	Time
Eri					
1	JA	4.54	-	JA	5.57
2	JA	0.25	*	*	*
3	JA	0.08	-	JA	0.29
4	Blanc	0.15	1	JA	3.13
5	JA	2.22	2	JA	5.10
6	JA	0.15	-	JA	0.35
Ws					
7	Blanc	3.07	2	Blanc	4.45
8	JA	1.34	-	JA	2.36
9	NR	*	*	*	*
10	JA	1.07	-	JA	1.26
11	JA	0.26	-	JA	0.49
12	JA	1.32	-	JA	1.52
Eri JA	**Vs Ws JA**				
13	Ws	0.14	3	Eri	3.32
14	Ws	0.08	2	Ws	8.48
15	Eri	0.32	1	Eri	2.01
16	Ws	0.25	-	Ws	0.45
17	Eri	2.27	-	Eri	2.48
18	Ws	0.17	1	Eri	1.12
19	Ws	0.22	-	Ws	1.31
20	Eri	2.18	-	Eri	3.10
21	Ws	0.07	4	Ws	6.02
22	Ws	3.36	4	*	*
23	Eri	020	-	Eri	1.19
24	Ws	0.18	-	Ws	0.37

Weight: Ws JA: 0.82/0.79/0.89/0.75
Eri JA: 0.80/0.79/0.88/0.75

Combination Ws vs. Cvi Th 17/01/08

Wasp	First choice	Time	Switches	Final choice	Time
Ws					
1	Blanc	0.17	-	Blanc	0.41
2	Blanc	0.08	2	Blanc	2.36
3	Blanc	1.48	1	JA	3.22
4	NR	*	*	*	*
5	Blanc	0.07	1	JA	1.04
6	JA	0.02	-	JA	2.09
Cvi					
7	JA	2.08	1	Blanc	3.57
8	JA	0.18	2	JA	4.22
9	JA	0.15	-	JA	0.51
10	JA	0.47	-	JA	1.57
11	Blanc	4.13	-	Blanc	7.37
12	JA	0.52	-	JA	2.48
Cvi JA	**Vs. Ws JA**				
13	Cvi	0.23	2	Cvi	3.26
14	Ws	0.36	1	*	*
15	NR	*	*	*	*
16	Ws	1.12	2	Ws	3.03
17	NR	*	*	*	*
18	Cvi	0.24	-	Cvi	0.45
19	Cvi	0.12	-	Cvi	0.52
20	NR	*	*	*	*
21	Cvi	1.07	1	Ws	3.02
22	Cvi	0.05	1	Ws	2.21
23	NR	*	*	*	*
24	Ws	0.48	-	Ws	1.13

Weight: Cvi JA: 0.86/0.83/0.83/0.90
Ws JA: 0.75/0.75/0.80/0.85

Combination Kond vs. Ws Fr 18/01/08

Wasp	First choice	Time	Switches	Final choice	Time
Kond					
1	Blanc	0.21	4		
2	Blanc	2.18	-		
3	JA	0.26	6		
4	JA	0.55	-		
5	JA	1.41	-		
6	JA	0.16	-		
Ws					
7	JA	0.20	-		
8	JA	0.38	4		
9	Blanc	3.42	1		
10	Blanc	0.48	-		
11	Blanc	0.10	2		
12	Blanc	1.30	1		
Kond JA	**Vs. Ws JA**				
13	Kond	1.44	*		
14	Kond	2.41	-		
15	Kond	3.19	-		
16	Ws	0.14	-		
17	Ws	0.38	-		
18	Ws	0.40	-		
19	Kond	1.52	3		
20	Kond	0.26	-		
21	Ws	2.03	-		
22	Ws	0.38	1		
23	Ws	0.23	-		
24	Kond	2.01	-		

Weight: Ws JA: 0.79/0.75/0.74/0.67
Kond JA: 0.71/1.02/0.89/1.03

Combination Kond vs. Ws We 30/01/2008

Wasp	First choice	Time	Switches	Final choice	Time
Kond					
1	JA	2.53	1	*	*
2	Blanc	0.04	-	Blanc	0.23
3	JA	1.17	1	Blanc	4.42
4	JA	0.59	-	JA	1.47
5	NR	*	*	*	*
6	JA	0.12	-	JA	2.46
Ws					
7	JA	1.39	*	*	*
8	JA	0.18	2	JA	3.00
9	JA	0.05	-	JA	0.5
10	JA	2.29	-	JA	2.59
11	Blanc	1.05	-	Blanc	4.09
12	Blanc	2.15	-	blanc	3.11
Kond JA	**Vs. Ws JA**				
13	Kond	0.06	*	*	*
14	Kond	0.16	2	Kond	3.05
15	Kond	0.15	3	Ws	2.57
16	Kond	1.07	2	*	*
17	Ws	1.17	-	Ws	1.39
18	Ws	1.38	*	*	*
19	Ws	0.12	-	Ws	0.32
20	Kond	0.24	-	Kond	1.07
21	Kond	0.05	-	Kond	1.27
22	Ws	0.45	-	Ws	3.32
23	Kond	0.08	-	Kond	0.28
24	Kond	0.11	-	Kond	0.37

Weight: Kond Blanc: 0.67/0.51/0.57/0.54
Ws JA: 0.73/0.61/0.68/0.64
Kond JA: 0.55/0.74/0.73/0.53

Combination Ws vs. Eri Th 31/01/2008

Wasp	First choice	Time	Switches	Final choice	Time
Ws					
1	JA	0.42	1	Blanc	2.36
2	Blanc	2.20	3	JA	8.39
3	JA	0.12	7	Blanc	8.19
4	JA	0.17	2	JA	7.24
5	JA	0.19	-	JA	3.55
6	JA	0.44	*	*	*
Eri					
7	JA	0.18	-	JA	1.25
8	NR	*	*	*	*
9	JA	0.33	2	JA	4.05
10	Blanc	2.03	-	Blanc	2.57
11	Blanc	1.14	-	Blanc	3.52
12	Blanc	2.35	2	Blanc	5.11
Ws JA	**Vs. Eri JA**				
13	NR	*	*	*	*
14	Eri	1.33	3	Ws	6.18
15	Ws	0.08	2	Ws	3.26
16	Ws	0.12	-	Ws	5.37
17	Eri	4.13	-	Eri	5.28
18	Eri	0.04	*	*	*
19	Eri	1.40	3	Ws	5.32
20	Eri	0.10	5	Ws	4.30
21	Ws	3.37	4	*	*
22	NR	*	*	*	*
23	Ws	0.10	3	Eri	3.54
24	NR	*	*	*	*

Weight: Cvi Blanc: 0.42/0.38/0.38/0.36
Kond Blanc: 0.38/0.52/0.73/0.64
Cvi JA: 0.37/0.48/0.45/0.46
Kond JA: 0.86/0.71/0.84/0.79

Combination Eri vs. Cvi Tu 19/02/2008

Wasp	First choice	Time	Switches	Final choice	Time
Cvi JA	**Vs. Eri JA**				
1	Eri	1.09	-	Eri	1.40
2	Eri	4.50	-	Eri	5.43
3	Eri	1.39	-	Eri	2.01
4	Eri	0.23	-	Eri	2.06
5	Eri	0.26	-	Eri	1.23
6	Eri	0.35	2	Eri	3.19
7	Eri	4.50	*	*	*
8	NR	*	*	*	*
9	Eri	1.30	-	Eir	1.56
10	Eri	3.51	-	Eri	5.24
11	NR	*	*	*	*
12	Eri	0.31	-	Eri	2.18
13	JA	4.57	-	JA	5.27
14	NR	*	*	*	*
15	Blanc	0.20	2	Blanc	2.40
16	JA	0.28	-	JA	0.51
17	JA	0.38	4	JA	6.53
18	JA	0.11	-	JA	3.09
19	JA	0.22	-	JA	0.46
20	JA	0.12	-	JA	0.40
21	Blanc	2.34	1	JA	4.11
22	JA	1.36	-	JA	1.56
23	JA	0.11	1	Blanc	1.19
24	JA	0.21	-	JA	8.12

Remark: 2 weeks old *D. semiclausum*

Weight: Eri JA: 0.69/1.10/0.92/1.05
Eri bl: 0.90/1.05/0.93/0.97
Cvi JA: 1.21/0.79/0.86/0.88
Cvi bl: 0.93/0.83/0.63/0.80

Combination Cvi vs. Col0 Th 21/02/08

Wasp	First choice	Time	Switches	Final choice	Time
Cvi JA	**vs. Col0 JA**				
1	Col0	3.48	-	Col0	5.43
2	Col0	0.55	2	Col0	6.18
3	Cvi	0.24	1	Col0	2.42
4	Cvi	1.40	4	Cvi	7.30
5	Col0	1.29	2	*	*
6	Col0	0.08	1	Cvi	1.54
7	Col0	3.52	-	Col0	4.45
8	Col0	1.46	-	Col0	3.16
9	Col0	1.23	-	Col0	1.44
10	Col0	0.52	2	Col0	2.19
11	NR	*	*	*	*
12	NR	*	*	*	*

Remark: not complete because of lack of *D. semiclausum*.

Weight: Cvi JA: 0.93/0.94/0.89/1.07
Col0 JA: 1.17/1.09/0.92/0.77

Combination Ws vs. Col0 Tu 26/02/08

Wasp	First choice	Time	Switches	Final choice	Time
Ws JA	**vs. Col0 JA**				
1	Ws	1.11	-	Ws	1.34
2	Ws	0.14	-	Ws	0.38
3	Ws	0.37	-	Ws	1.37
4	Col0	0.19	-	Col0	0.38
5	Ws	0.46	-	Ws	1.05
6	Ws	0.09	-	Ws	0.29
7	Col0	4.20	-	Col0	9.30
8	Col0	1.58	4	Col0	9.23
9	Ws	1.17	-	Ws	1.57
10	Ws	2.12	-	Ws	2.40
11	Col0	0.28	-	Col0	3.01
12	Col0	0.03	-	Col0	2.47
Ws					
13	Blanc	1.16	1	JA	4.17
14	Blanc	0.49	5	JA	5.58
15	JA	0.50	1	*	*
16	JA	0.13	-	JA	0.31
17	JA	0.16	-	JA	1.55
18	JA	0.31	-	JA	0.57
Col0					
19	JA	2.09	-	JA	6.31
20	JA	0.51	1	Blanc	3.11
21	Blanc	0.03	-	Blanc	1.51
22	JA	1.11	2	JA	7.14
23	JA	0.39	-	JA	1.11
24	JA	2.33	6	JA	8.11

Weight: Ws JA: 1.30/1.30/1.26/1.37
Col0 JA: 0.90/0.97/0.77/0.94

Combination Eri vs. Col0 We 27/02/08

Wasp	First choice	Time	Switches	Final choice	Time
Eri JA	**vs. Col0 JA**				
1	Col0	0.19	4	Col0	3.24
2	Col0	0.27	-	Col0	0.57
3	Col0	0.20	-	Col0	0.53
4	Col0	0.09	-	Col0	0.33
5	Eri	1.04	1	Col0	3.05
6	Col0	0.30	-	Col0	1.05
7	Eri	2.50	1	*	*
8	Col0	0.52	-	Col0	2.29
9	Col0	1.20	-	Col0	5.25
10	Eri	1.30	2	Eri	4.34
11	Col0	0.26	1	Eri	1.38
12	Col0	0.55	4	Col0	8.22
Col0					
13	JA	0.34	2	JA	5.02
14	Blanc	0.51	-	Blanc	1.58
15	JA	0.12	3	*	*
16	JA	0.21	-	JA	0.43
17	JA	0.12	1	Blanc	2.43
18	JA	1.14	2	JA	4.08
Eri					
19	JA	2.42	7	Blanc	7.30
20	JA	0.14	*	*	*
21	Blanc	2.46	1	JA	5.11
22	Blanc	2.00	1	JA	9.31
23	Blanc	0.57	3	JA	3.15
24	JA	1.17	-	JA	1.34

Weight: Col0 JA: 0.74/0.78/0.80/0.58
Eri JA: 0.73/0.69/0.84/0.64

Combination Ws vs. Col0 Th 28/02/08

Wasp	First choice	Time	Switches	Final choice	Time
Ws JA	**Vs. Col0 JA**				
1	Ws	0.25	-	Ws	4.47
2	NR	*	*	*	*
3	NR	*	*	*	*
4	Ws	0.51	-	Ws	4.08
5	NR	*	*	*	*
6	Col0	1.22	*	*	*
7	Ws	0.58	1	Col0	2.31
8	Col0	0.15	1	Ws	1.21
9	Ws	0.43	2	*	*
10	NR	*	*	*	*
11	Col0	0.10	-	Col0	0.33
12	Col0	0.26	-	Col0	1.20
Col0					
13	Blanc	2.31	-	Blanc	4.36
14	NR	*	*	*	*
15	Blanc	0.57	1	*	*
16	JA	0.35	1	Blanc	3.17
17	Blanc	0.55	1	JA	6.58
18	JA	3.18	*	*	*
Ws					
19	Blanc	0.08	1	JA	3.11
20	JA	1.46	2	JA	6.24
21	JA	1.39	-	JA	2.09
22	JA	1.04	-	JA	1.32
23	Blanc	4.20	-	Blanc	7.19
24	JA	0.24	-	JA	0.55

Weight: Ws JA; 0.60/0.54/0.50/0.52
Col0 JA: 0.59/0.56/0.58/0.52

Combination Eri vs. Col0 Mo 03/03/08

Wasp	First choice	Time	Switches	Final choice	Time
Eri JA	**vs. Col0 JA**				
1	Col0	3.36	1	Eri	4.55
2	NR	*	*	*	*
3	Eri	0.57	1	Col0	3.06
4	Eri	0.10	-	Eri	0.31
5	Eri	2.00	1	Col0	7.41
6	Eri	0.45	3	Col0	1.35
7	Eri	0.24	2	Eri	2.24
8	NR	*	*	*	*
9	Eri	0.39	-	Eri	3.25
10	Eri	2.32	1	Col0	4.12
11	Col0	0.49	2	Col0	4.46
12	NR	*	*	*	*
Eri					
13	JA	0.08	-	JA	0.33
14	Blanc	0.12	1	JA	0.58
15	NR	*	*	*	*
16	JA	2.54	-	JA	4.47
17	JA	0.38	2	JA	5.19
18	JA	0.37	*	*	*
Col0					
19	NR	*	*	*	*
20	JA	0.40	-	JA	1.01
21	JA	1.53	-	JA	2.21
22	NR	*	*	*	*
23	JA	0.13	-	JA	0.39
24	Blanc	0.36	-	Blanc	0.58

Weight: Col0 JA: 1.11/1.31/0.89/1.00
Eri JA: 1.17/0.87/1.23/1.12

Combination Cvi vs. Col0 Tu 04/03/08

Wasp	First choice	Time	Switches	Final choice	Time
Cvi JA	**Vs. Col0 JA**				
1	Col0	0.23	-	Col0	0.42
2	Cvi	0.22	-	Cvi	1.02
3	Col0	1.50	2	Col0	5.19
4	Cvi	0.12	-	Cvi	3.18
5	Col0	1.01	4	*	*
6	Col0	2.07	-	Col0	6.08
7	Col0	0.15	-	Col0	0.47
8	Cvi	3.47	3	Col0	6.48
9	Col0	1.48	-	Col0	3.20
10	Col0	1.43	-	Col0	3.31
11	Col0	1.30	-	Col0	3.09
12	Col0	0.12	-	Col0	0.30
Col0					
13	JA	2.05	-	JA	2.35
14	Blanc	0.20	-	Blanc	0.58
15	Blanc	0.12	-	Blanc	9.17
16	JA	1.03	1	Blanc	3.03
17	JA	0.24	-	JA	5.39
18	NR	*	*	*	*
Cvi					
19	JA	0.16	-	JA	1.49
20	JA	0.09	-	JA	2.51
21	JA	0.21	*	*	*
22	NR	*	*	*	*
23	Blanc	1.34	1	JA	2.32
24	JA	0.29	-	JA	0.56

Weight: Col0 JA: 1.07/0.84/1.10/1.07
Cvi JA: 0.85/0.92/1.12/0.90

Combination Eri vs. Col0 We 05/03/08

Wasp	First choice	Time	Switches	Final choice	Time
Eri JA	**vs. Col0 JA**				
1	NR	*	*	*	*
2	Col0	0.16	*	*	*
3	Col0	0.09	-	Col0	0.35
4	Col0	0.07	1	*	*
5	Eri	0.29	-	Eri	1.29
6	Col0	2.19	1	Eri	4.18
7	Col0	0.50	-	Col0	1.10
8	NR	*	*	*	*
9	NR	*	*	*	*
10	NR	*	*	*	*
11	Col0	0.45	3	Eri	3.08
12	Eri	0.23	-	Eri	7.10
Col0					
13	Blanc	0.44	*	*	*
14	JA	0.42	*	*	*
15	JA	0.20	1	Blanc	1.50
16	JA	0.07	-	JA	0.29
17	JA	4.50	-	JA	6.13
18	Blanc	2.00	2	Blanc	6.44
Eri					
19	JA	0.18	3	Blanc	1.58
20	JA	0.24	-	JA	0.40
21	JA	4.47	1	Blanc	6.38
22	Blanc	0.25	-	Blanc	0.46
23	Blanc	0.19	-	Blanc	3.00
24	Blanc	1.26	2	Blanc	4.29

Weight: Eri JA: 1.31/1.09/1.27/1.32
Col0 JA: 1.07/0.95/1.49/1.03

Combination Cvi vs. Col0 Th 06/03/08

Wasp	First choice	Time	Switches	Final choice	Time
Cvi JA	**Vs. Col0 JA**				
1	Col0	0.12	-	Col0	0.34
2	Cvi	0.18	-	Cvi	0.57
3	Col0	0.20	-	Col0	0.41
4	Col0	0.29	-	Col0	0.52
5	Col0	0.30	-	Col0	1.42
6	Col0	0.49	-	Col0	1.16
7	Col0	1.24	-	Col0	3.28
8	Col0	1.17	1	*	*
9	Cvi	1.15	3	Col0	5.19
10	NR	*	*	*	*
11	Col0	1.29	-	Col0	8.01
12	Col0	1.13	*	*	*
Cvi					
13	Blanc	0.18	1	JA	6.21
14	JA	3.01	-	JA	5.08
15	NR	*	*	*	*
16	Blanc	1.19	2	Blanc	6.10
17	JA	3.56	-	JA	4.38
18	JA	4.22	-	JA	5.15
Col0					
19	NR	*	*	*	*
20	JA	0.26	1	Blanc	1.24
21	Blanc	1.31	-	Blanc	1.50
22	JA	0.26	2	JA	5.27
23	Blanc	1.26	-	Blanc	2.56
24	JA	0.17	-	JA	3.51

Weight: Cvi JA; 0.96/0.88/1.06/1.03
Col0 JA: 1.26/1.40/0.85/1.07
Col0 Bl: 1.9/1.57/1.18/1.56

Combination Eri vs. Col0 Fr 07/03/08

Wasp	First choice	Time	Switches	Final choice	Time
Eri JA	**vs. Col0 JA**				
1	NR	*	*	*	*
2	Eri	0.50	1	Col0	2.05
3	Eri	4.22	*	*	*
4	Col0	1.13	1	Eri	2.26
5	Col0	0.07	-	Col0	0.28
6	Col0	2.29	*	*	*
7	Eri	2.45	1	Col0	3.36
8	Col0	4.45	-	Col0	8.20
9	Eri	1.01	3	*	*
10	NR	*	*	*	*
11	Col0	0.12	-	Col0	0.58
12	Eri	3.59	*	*	*
Col0					
13	NR	*	*	*	*
14	Blanc	0.13	-	Blanc	0.33
15	JA	0.48	-	JA	8.09
16	JA	0.13	-	JA	2.36
17	Blanc	2.48	-	Blanc	6.04
18	JA	1.17	2	JA	5.41
Eri					
19	JA	1.32	-	JA	1.53
20	NR	*	*	*	*
21	JA	0.12	-	JA	6.09
22	Blanc	1.04	5	JA	5.55
23	Blanc	0.27	-	Blanc	1.22
24	Blanc	0.58	-	Blanc	1.56

Weight: Col0 JA: 0.93/1.15/0.87/1.21
Eri JA: 1.40/1.55/1.41/1.00

Combination Ler vs. Col0 We 12/03/08

Wasp	First choice	Time	Switches	Final choice	Time
Ler JA	**Vs. Col0 JA**				
1	Ler	0.12	-	Ler	1.56
2	NR	*	*	*	*
3	Ler	0.22	-	Ler	1.59
4	Col0	0.13	-	Col0	0.29
5	Ler	0.10	-	Ler	1.40
6	Ler	0.39	-	Ler	1.56
7	Ler	0.14	-	Ler	0.32
8	Ler	0.22	1	Col0	4.02
9	Ler	0.12	-	Ler	0.28
10	Col0	1.34	-	Col0	2.03
11	Ler	2.48	-	Ler	4.50
12	Ler	3.11	-	Ler	3.34
Ler					
13	JA	0.15	-	JA	0.37
14	JA	0.28	-	JA	1.27
15	JA	3.28	-	JA	4.30
16	JA	0.15	-	JA	1.00
17	JA	0.12	1	Blanc	4.09
18	JA	0.35	-	JA	1.01
Col0					
19	JA	0.04	-	JA	0.20
20	JA	0.39	-	JA	1.02
21	JA	0.08	-	JA	0.27
22	JA	0.56	-	JA	1.22
23	Blanc	1.20	-	Blanc	1.52
24	JA	0.35	-	JA	7.18

Weight: Ler JA; 0.52/0.55/0.55/0.49
Ler Bl:0.63/0.40/0.52/0.46
Col0 JA: 0.55/0.54/0.48/0.57
Col0 Bl: 0.51/0.52/0.55/0.52

Combination Ler vs. Col0 Th 13/03/08

Wasp	First choice	Time	Switches	Final choice	Time
Ler JA	**Vs. Col0 JA**				
1	Ler	0.13	1	Col0	5.38
2	Ler	0.38	-	Ler	0.53
3	Ler	0.10	1	Col0	2.18
4	Ler	1.35	1	Col0	4.46
5	Col0	0.45	-	Col0	4.51
6	Col0	0.12	1	*	*
7	Ler	0.08	-	Ler	0.26
8	Ler	0.12	-	Ler	0.31
9	Ler	1.41	1	Col0	2.40
10	Ler	0.15	4	Ler	3.59
11	Ler	0.18	-	Ler	1.22
12	Ler	0.30	-	Ler	8.08
Col0					
13	JA	1.25	-	JA	1.52
14	Blanc	0.50	-	Blanc	6.08
15	NR	*	*	*	*
16	JA	0.18	-	JA	1.08
17	JA	1.10	-	JA	4.34
18	NR	*	*	*	*
Ler					
19	JA	0.19	-	JA	0.44
20	JA	0.10	-	JA	0.48
21	JA	0.12	-	JA	0.35
22	JA	3.25	-	JA	4.33
23	JA	0.34	-	JA	5.30
24	JA	3.00	-	JA	5.19

Weight: Ler JA; 0.55/0.48/0.46/0.53
Ler Bl: 0.57/0.50/0.53/0.63
Col0 JA: 0.57/0.67/0.62/0.53
Col0 Bl: 0.60/0.60/0.56/0.51

Combination Ler vs. Col0 Fr 14/03/08

Wasp	First choice	Time	Switches	Final choice	Time
Ler JA	**Vs. Col0 JA**				
1	Ler	2.43	-	Ler	3.19
2	Col0	2.36	4	*	*
3	NR	*	*	*	*
4	Ler	0.12	-	Ler	0.34
5	Ler	0.15	-	Ler	0.36
6	Ler	2.41	2	Ler	5.19
7	Col0	0.36	1	Ler	9.21
8	Ler	0.45	1	Col0	1.23
9	Ler	0.03	-	Ler	0.49
10	Ler	0.09	-	Ler	0.37
11	Ler	1.21	-	Ler	1.43
12	Ler	1.07	-	Ler	1.38
Ler					
13	JA	2.26	-	JA	6.51
14	JA	0.54	-	JA	2.04
15	JA	2.19	-	JA	2.44
16	NR	*	*	*	*
17	JA	4.16	-	JA	4.40
18	JA	2.41	1	Blanc	7.14
Col0					
19	NR	*	*	*	*
20	NR	*	*	*	*
21	JA	2.52	5	Blanc	9.14
22	Blanc	0.07	-	Blanc	2.44
23	JA	2.20	-	JA	2.56
24	Blanc	1.48	-	Blanc	2.31

Weight: Ler JA; 0.62/0.61/0.55/0.64
Ler Bl: 0.47/0.62/0.56/0.51
Col0 JA: 0.60/1.01/0.64/0.62
Col0 Bl: 0.53/0.59/0.64/0.53

Combination Ler vs. Col0 Tu 18/03/08

Wasp	First choice	Time	Switches	Final choice	Time
Ler JA	**Vs. Col0 JA**				
1	Ler	0.36	-	Ler	1.27
2	NR	*	*	*	*
3	Ler	1.01	-	Ler	1.22
4	Ler	3.30	-	Ler	3.51
5	Col0	1.19	1	Ler	3.27
6	Col0	0.55	1	Ler	2.05
7	Col0	2.45	*	*	*
8	Col0	1.41	-	Col0	2.16
9	Ler	0.11	1	*	*
10	Ler	2.40	-	Ler	6.47
11	Col0	1.13	-	Col0	2.51
12	Ler	0.38	1	Col0	3.04
Col0					
13	JA	0.28	2	JA	7.00
14	JA	0.29	2	*	*
15	Blanc	0.21	1	JA	3.01
16	NR	*	*	*	*
17	JA	0.51	2	JA	3.47
18	JA	3.02	*	*	*
Ler					
19	Blanc	0.38	-	Blanc	0.57
20	JA	1.13	-	JA	1.43
21	JA	0.09	-	JA	0.28
22	JA	3.26	-	JA	4.35
23	Blanc	0.20	-	Blanc	2.07
24	JA	4.02	2	JA	5.39

Weight: Ler JA; 0.85/0.85/0.75/1.15
Ler Bl: 0.72/0.96/0.75/0.91
Col0 JA: 0.93/0.94/0.84/0.91
Col0 Bl: 0.86/0.91/0.92/0.82

Combination Ws vs. Col0 We 19/03/08

Wasp	First choice	Time	Switches	Final choice	Time
Ws JA	**Vs. Col0 JA**				
1	NR	*	*	*	*
2	NR	*	*	*	*
3	Col0	0.34	1	*	*
4	Ws	0.55	2	*	*
5	NR	*	*	*	*
6	Ws	0.19	1	Col0	2.20
7	Col0	0.02	-	Col0	0.22
8	Ws	0.40	-	Ws	1.00
9	NR	*	*	*	*
10	Col0	3.19	-	Col0	4.40
11	NR	*	*	*	*
12	NR	*	*	*	*
Col0					
13	Blanc	2.05	1	JA	3.17
14	JA	0.22	-	JA	1.44
15	JA	0.41	-	JA	1.12
16	Blanc	2.50	-	Blanc	3.52
17	JA	0.25	1	Blanc	1.29
18	Blanc	0.08	1	JA	0.41
Ws					
19	JA	1.57	-	JA	9.34
20	JA	3.13	*	*	*
21	JA	0.55	-	JA	1.55
22	JA	0.20	-	JA	0.41
23	JA	0.42	-	JA	5.27
24	JA	1.41	2	JA	4.11

Weight: Ws JA; 0.96/0.97/1.02/1.04
Ws Bl: 1.06/0.93/0.98/1.31
Col0 JA: 0.98/0.97/1.06/1.01
Col0 Bl: 1.06/0.72/0.84/0.82

Combination Cvi vs. Col0 Th 20/03/08

Wasp	First choice	Time	Switches	Final choice	Time
Cvi JA	**Vs. Col0 JA**				
1	Cvi	0.39	-	Cvi	1.06
2	Col0	0.13	-	Col0	0.45
3	Col0	0.18	-	Col0	0.40
4	NR	*	*	*	*
5	Col0	2.47	-	Col0	3.07
6	Col0	0.18	-	Col0	0.48
7	Cvi	0.21	-	Cvi	3.53
8	Cvi	0.17	1	Col0	2.42
9	Cvi	1.36	1	*	*
10	Cvi	1.18	1	Col0	2.42
11	Cvi	1.04	-	Cvi	1.24
12	Cvi	0.47	1	Col0	2.42
Col0					
13	NR	*	*	*	*
14	JA	0.17	-	JA	0.36
15	Blanc	0.11	-	Blanc	0.38
16	JA	1.52	2	*	*
17	Blanc	0.39	-	Blanc	1.46
18	JA	0.08	-	JA	0.28
Cvi					
19	Blanc	0.08	-	Blanc	2.21
20	JA	0.13	-	JA	0.33
21	JA	0.39	-	JA	2.35
22	Blanc	0.32	-	Blanc	1.42
23	JA	2.43	*	*	*
24	JA	1.25	-	JA	6.37

Weight: Cvi JA; 1.08/1.14/0.88/1.00
Cvi Bl:1.04/1.10/0.90/0.96
Col0 JA: 1.34/1.08/0.96/1.49
Col0 Bl: 0.76/0.71/0.89/0.82

Combination Kond vs. Col0 Fr 28/03/08

Wasp	First choice	Time	Switches	Final choice	Time
KondJA	**Vs. Col0 JA**				
1	NR	*	*	*	*
2	Col0	0.30	1	Kond	2.32
3	Col0	4.34	-	Col0	7.33
4	Col0	0.07	-	Col0	2.25
5	Col0	1.46	-	Col0	2.12
6	Kond	1.16	3	*	*
7	Kond	0.23	-	Kond	0.44
8	Col0	0.10	4	Col0	5.46
9	Col0	1.50	-	Col0	2.28
10	Kond	0.15	-	Kond	5.52
11	Kond	0.39	-	Kond	1.07
12	Kond	1.36	2	Kond	5.24
Col0					
13	NR	*	*	*	*
14	JA	2.35	2	JA	9.06
15	JA	0.24	-	JA	0.44
16	JA	0.34	-	JA	3.57
17	Blanc	0.47	1	JA	2.42
18	Blanc	1.58	4	*	*
Kond					
19	Blanc	0.51	-	Blanc	1.19
20	JA	4.02	2	*	*
21	JA	2.13	4	JA	7.37
22	JA	0.10	-	JA	1.15
23	JA	2.25	*	*	*
24	Blanc	2.16	-	Blanc	3.01

Weight: Kond JA; 0.39/0.45/0.40/0.39
Kond Bl: 0.37/0.34/0.41/0.40
Col0 JA: 0.39/0.42/0.47/0.39
Col0 Bl: 0.31/0.46/0.43/0.42

Combination Kond vs. Col0 Tu 01/04/08

Wasp	First choice	Time	Switches	Final choice	Time
KondJA	**Vs. Col0 JA**				
1	Col0	2.21	-	Col0	4.51
2	Kond	0.26	-	Kond	1.12
3	Kond	1.15	-	Kond	1.37
4	Kond	1.01	-	Kond	4.39
5	Kond	0.42	-	Kond	1.31
6	Kond	0.19	-	Kond	0.43
7	Kond	0.27	-	Kond	0.47
8	Kond	0.16	-	Kond	0.37
9	Kond	1.04	2	Kond	2.53
10	Kond	3.53	-	Kond	7.05
11	Kond	0.47	-	Kond	1.09
12	Kond	0.05	-	Kond	0.22
Kond					
13	Blanc	0.31	-	Blanc	3.08
14	JA	1.14	-	JA	2.11
15	JA	0.49	-	JA	1.40
16	Blanc	0.08	-	Blanc	0.26
17	Blanc	2.03	-	Blanc	2.21
18	Blanc	0.30	-	Blanc	0.54
Col0					
19	JA	3.35	1	*	*
20	Blanc	0.24	4	Blanc	8.07
21	Blanc	3.07	3	JA	7.39
22	NR	*	*	*	*
23	Blanc	1.37	-	Blanc	1.57
24	JA	0.37	3	Blanc	5.50

Weight: Kond JA; 0.95/0.96/0.93/0.94
Kond Bl: 0.90/0.89/0.76/0.79
Col0 JA: 0.89/0.79/0.80/0.78
Col0 Bl: 0.82/0.80/0.81/0.83

Combination Kond vs. Col0 We 02/04/08

Wasp	First choice	Time	Switches	Final choice	Time
KondJA	**Vs. Col0 JA**				
1	Kond	1.12	2	*	*
2	Kond	0.35	-	Kond	1.02
3	Kond	0.20	1	Col0	1.47
4	Kond	1.08	-	Kond	1.44
5	Kond	0.43	-	Kond	1.03
6	Kond	0.40	8	Kond	8.31
7	NR	*	*	*	*
8	Kond	0.29	-	Kond	1.29
9	Kond	0.26	-	Kond	7.27
10	Col0	0.15	1	Kond	1.00
11	Kond	0.14	-	Kond	0.46
12	Kond	0.12	-	Kond	0.33
Col0					
13	NR	*	*	*	*
14	Blanc	1.19	2	Blanc	4.26
15	Blanc	0.07	1	JA	1.32
16	Blanc	1.34	1	JA	3.21
17	JA	0.21	-	JA	4.22
18	Blanc	0.37	-	Blanc	1.04
Kond					
19	Blanc	0.25	-	Blanc	2.11
20	Blanc	0.12	-	Blanc	0.33
21	Blanc	0.37	-	Blanc	1.01
22	JA	0.34	-	JA	1.05
23	JA	0.15	-	JA	0.39
24	JA	2.46	*	*	*

Weight: Col0 JA; 0.88/0.95/0.76/1.04
Col0 Bl: 0.99/0.64/1.06/0.97
Kond JA: 1.05/1.10/1.03/0.98
Kond Bl: 1.10/0.91/1.21/1.20

Combination Eri vs. Cvi Th 03/04/08

Wasp	First choice	Time	Switches	Final choice	Time
EriJA	**Vs. Cvi JA**				
1	Cvi	0.15	-	Cvi	0.33
2	Eri	0.04	-	Eri	0.33
3	NR	*	*	*	*
4	Cvi	3.52	-	Cvi	5.31
5	NR	*	*	*	*
6	Cvi	2.37	*	Cvi	5.07
7	NR	*	*	*	*
8	NR	*	*	*	*
9	Eri	0.15	-	Eri	0.35
10	Cvi	0.48	1	Eri	2.13
11	Eri	0.08	-	Eri	1.12
12	NR	*	*	*	*
Cvi					
13	JA	0.48	-	JA	6.05
14	Blanc	0.11	-	Blanc	3.56
15	NR	*	*	*	*
16	JA	0.50	-	JA	1.34
17	Blanc	2.22	-	Blanc	3.06
18	JA	0.20	-	JA	0.44
Eri					
19	JA	0.24	2	JA	2.18
20	JA	1.08	-	JA	3.08
21	JA	3.52	*	*	*
22	JA	1.51	-	JA	3.13
23	JA	0.16	-	JA	0.34
24	JA	3.52	-	JA	4.14

Weight: Cvi JA; 0.86/0.97/0.74/0.81
Cvi Bl:0.76/0.84/0.73/0.83
Eri JA: 1.22/1.24/1.05/1.19
Eri Bl: 1.60/1.12/0.91/1.17

Combination Kond vs. Col0 Tu 08/04/08

Wasp	First choice	Time	Switches	Final choice	Time
KondJA	**Vs. Col0 JA**				
1	Kond	0.16	-	Kond	0.56
2	Kond	0.12	-	Kond	0.57
3	Kond	0.11	-	Kond	0.40
4	Kond	0.15	-	Kond	0.36
5	Kond	2.01	-	Kond	4.55
6	Kond	0.05	-	Kond	0.26
7	Kond	0.22	1	Kond	2.16
8	Kond	0.35	-	Kond	0.58
9	Kond	0.46	-	Kond	2.27
10	Kond	1.48	-	Kond	2.55
11	Kond	0.14	-	Kond	0.37
12	Col0	0.30	1	Kond	1.46

Weight: Kond JA: 1.54/1.78/1.57/1.35
Col0 JA: 1.65/1.45/1.31/1.56