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**Chemical ecology of Cabbage White (*Pieris* sp.)**

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## 1. General introduction and aim of the thesis

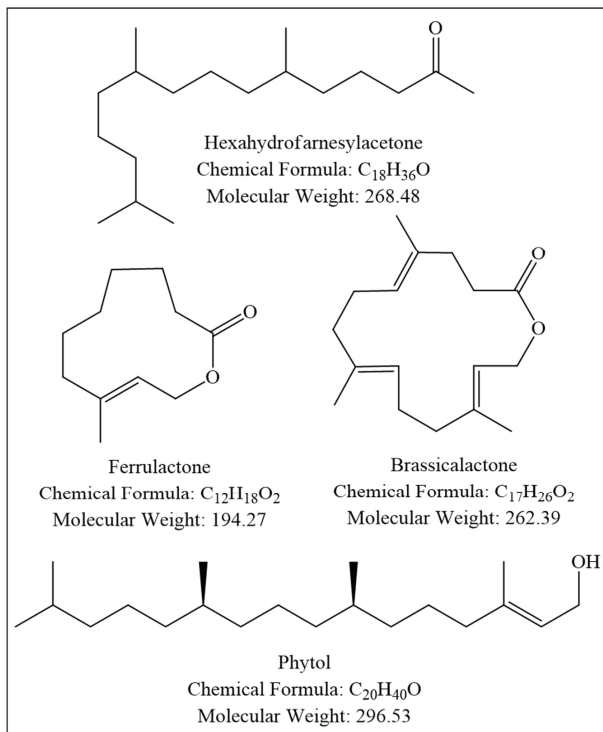
Chemical ecology explores the structure, origin, and function of naturally occurring chemicals that mediate intraspecific or interspecific interactions. These chemicals are known as semiochemicals. Depending on their function, they were recently divided into three major classes: pheromones, kairomones, and allomones (Drijfhout 2017). Wyatt explained pheromones as “molecules that are evolved signals, in defined ratios in the case of multiple component pheromones, which are emitted by an individual and received by a second individual of the same species, in which they cause a specific reaction, for example, a stereotyped behavior or a developmental process” (Wyatt 2010; modified after Karlson and Lüscher 1959).

Among Lepidoptera insects, butterflies are about 9% of the order worldwide, totaling about 20,400 described species. They are one of the most widespread and widely recognized insects (Heppner 2008; Powell and Opler 2009). They are currently grouped within the taxonomic superfamily Papilionoidea, with seven families, namely Papilionidae, Pieridae, Riodinidae, Lycaenidae, Nymphalidae, Hesperidae (skippers), and Hedyliidae (Van Nieukerken et al. 2011). In insects like Lepidoptera, the existence of communication via pheromone is widespread and widely discussed. Many different types of pheromones have been identified such as sex pheromones (Boeckh and Ernst 1987; Kanzaki et al. 1992; Hildebrand and Shepherd 1997) and social pheromones (Hölldobler and Wilson 1990; Mizunami et al. 2010; Verheggen et al. 2010).

Butterflies are day-flying insects (excluding hedyliids) whose partner-finding strategy is mainly based on visual and olfactory cues. The female butterflies do not have sex pheromone glands in their ovipositor, unlike moths. Therefore, they do not release long-range pheromones to find their partners. Instead, the male butterflies used their vision to detect conspecific females at some distance and to pursue them. The males release short-range pheromones to attract females, resulting in courtship (Silberglied 1984; Monteys et al. 2016). These short-range pheromones produced in the wing's scales are the bouquet of males, which help females in selecting their conspecifics before courtship.

The structure of wings' scales called androconia was found in Pierid species such as *P. brassicae*, *P. rapae*, and *P. napi*. Each Pierid species has characteristic pheromones produced in these androconia (Bergström and Lundgren 1973). In *P. rapae*, olfactory cues in recognition of partners and courtship behavior were studied and concluded that males could be attracted to females enclosed in Petri dishes (Obara 1964; Obara and Hidaka 1968). After visual cues, the smell was also reputed to play a part in courtship and attraction of imagines at close quarters to bring insects together (Hertz 1927). The compounds produced in male wings of *P. rapae* and *P. brassicae*,

known as aphrodisiac pheromone, have been identified (Yildizhan et al. 2009). These are ferrulactone, hexahydrofarnesylacetone, and E-phytol in *P. rapae*, while the presence of brassicalactone instead of ferrulactone in *P. brassicae* has been identified (figure 1.1).



**Figure 1.1:** Chemical Structures of pheromones of wings of the *Pieris* species (Yildizhan et al. 2009)

To ensure environment-friendly agricultural practices, chemicals and/or pesticides must be avoided. Therefore, it becomes essential to find modern environmentally safe methods to affect cabbage white pests. Biological control of Lepidoptera has traditionally relied on pathogens, such as viruses (Harcourt 1966; Biever and Wilkinson 1978) and nematodes (Peters 1996). Insect natural enemies such as predators and parasitoids have also been used (Lucas et al. 1998; van Driesche and Hoddle 1997; Harvey et al. 1999; Karowe and Schoonhoven 1992). Another important way of control can be found out by studying the chemical ecology of Lepidoptera. The components of the male sex pheromone of the African butterfly *Bicyclus anynana* (Butler) have been explored to be (Z)-9-tetradecenol (Z9-14: OH), hexadecanal (16: Ald), and 6,10,14-

trimethylpentadecan-2-ol (6,10,14-trime-15-2-ol). Male sex pheromones of queen butterfly, *Danaus gilippus* (Berenice), and a butterfly in the subfamily Danainae, *Idea leuconoe* (Erichson), have also been identified (Pliske and Eisner 1969; Nishida et al. 1996). Male sex pheromone of *Pieris napi* has also been reported about a decade ago (Andersson et al. 2007). One way to control the cabbage butterflies is using other IPM strategies such as pheromone traps and mass trapping, where the studies regarding insect pheromones are considered particularly important. Recently Mating disruption of *Cossus insularis* (Staudinger) (Lepidoptera: Cossidae) with a synthetic pheromone was tested and resulted in a significantly decreased percentage of damaged trees (Hoshi et al. 2016).

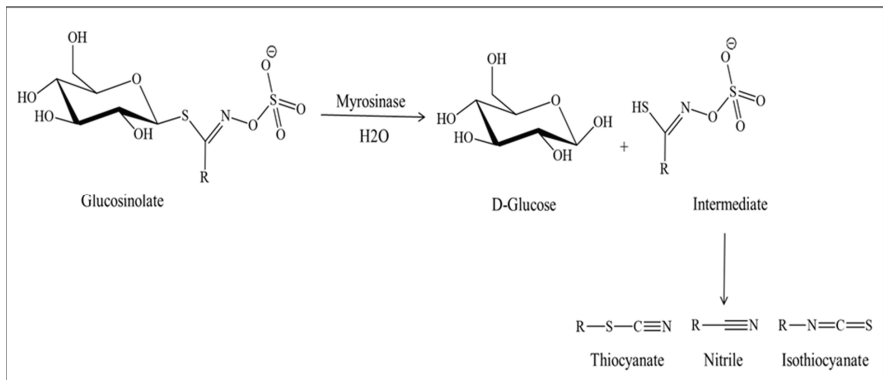
The second aspect of chemical ecology also deals with the study of defense compounds produced by host plants. Many *Brassica* species are considered host plants of *Pieris* species (Feltwell 1982) that produce secondary metabolites known as glucosinolates (GS). They have a characteristic chemical structure with a sulfur-linked  $\beta$ -D-glucopyranose moiety and an amino acid-derived side chain (figure 1.2). They are further divided based on the side chain into three groups, aliphatic, aromatic, and indole GS. A very comprehensive compilation of structures, trivial names, and distribution has been reviewed in which more than 140 GS have been listed (Fahey et al. 2001; European food safety authority 2008). GS-containing plants possess GS hydrolyzing enzyme, myrosinase, that is spatially separated from its substrate in the intact plant (Kelly et al. 1998). GS does not seem to be toxic themselves, but when they are brought together with myrosinase, they are rapidly hydrolyzed to toxic isothiocyanates (Cole 1976; Daxenbichler et al. 1977; Hanschen and Schreiner 2017) and some other compounds such as nitriles and epithionitriles (Halkier and Gershenzon 2006; Fenwick et al. 1983).

GS derivatives have a versatile range of functions. Some of them have a protective effect against cancer. Some GS derivatives may have detrimental effects, such as progoitrin and some derived from alkenyl GS. These compounds are mostly reported in rapeseed meals, which act as antinutrients affecting animal growth and development and lowering food intake (Rosa et al. 1997). For this study, four aliphatic and four indolic GS were chosen. Aliphatic GS includes 2-Hydroxy-3-butenyl, 2-Propenyl, 4-Methylsulfinylbutyl, and 3-Butenyl, while the indolic GS includes 4-Hydroxyindol-3-ylmethyl, Indol-3-ylmethyl, 4-Methoxyindol-3-ylmethyl, and 1-Methoxyindol-3-ylmethyl GS (figure 1.3).

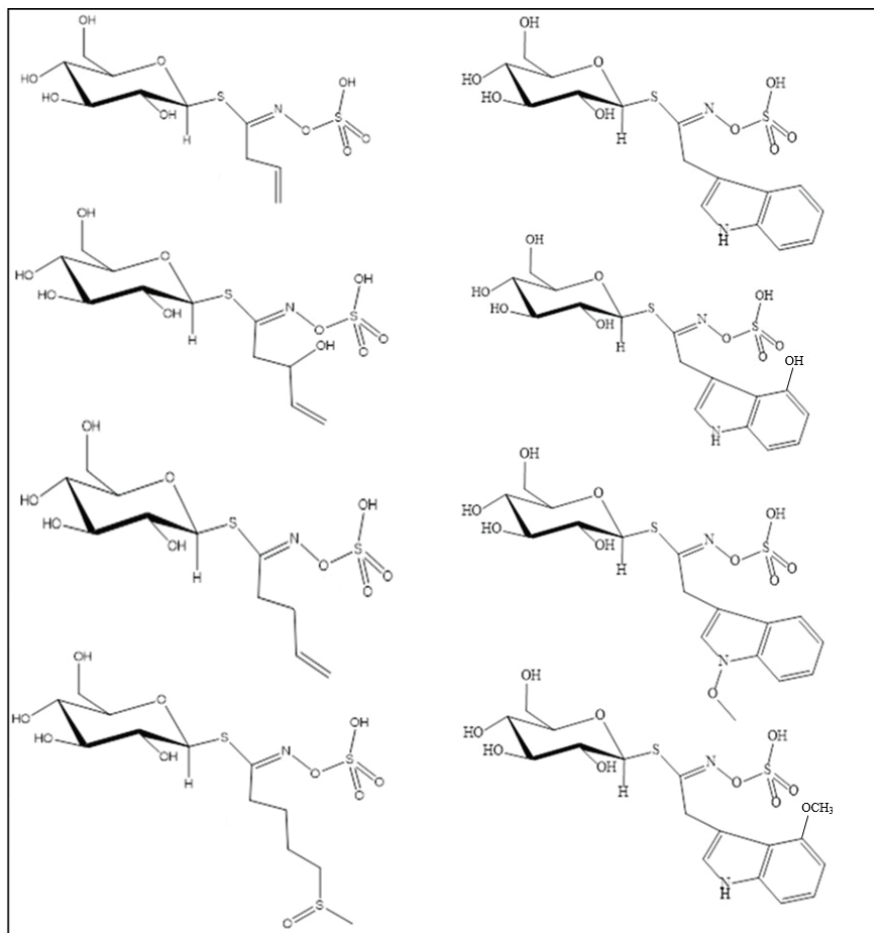
*P. rapae* and *P. brassicae* have been observed as oligophagous insects feeding upon a wide range of plants. They can feed on about 105 plant families. A total of 83 species of families, Brassicaceae,

Papilionaceae, Resedaceae, Capparaceae, and Tropaeolaceae, are considered principal families exploited by *Pieris* species (Feltwell 1982; Bhandari et al. 2009). The close relation between herbivorous insects and their host plants has been widely discussed for decades. Prof. L.M. Schoonhoven's research describes that this relationship is due to a highly specialized sensory system of the larva and imago and their responses to chemical substances (GS) produced by the plants (Schoonhoven 1967; 1969). The caterpillars of *Pieris* are specialist herbivores that feed only on plants in the order Capparales that produce GS (Harvey et al. 2010). The adult females and larvae of *P. rapae* and *P. brassicae* use GS in Brassica plants for host selection for oviposition and feeding (Renwick et al. 1992; van Loon et al. 1992; Moyes et al. 2000; Schoonhoven and van Loon 2002).

The feeding of *P. brassicae* and *P. rapae* larvae upon different parts such as flowers and leaves of *Brassica nigra* var. *abyssinica* A. Braun was studied. As a result of larval feeding, allyl GS (sinigrin) levels become higher than in undamaged plants (Smallegange et al. 2007). Aphids increased aliphatic GS content in *Arabidopsis thaliana*, but *P. rapae* did not cause a significant induction of aliphatic GS, while the indolyl GS content increased by about 20% (Mewis et al. 2006).



**Figure 1.2:** Products of GS hydrolysis



**Figure 1.3:** Structures of aliphatic and indolic GS considered in this study. Left upside down: 2-propenyl, 2-hydroxy-3-butenyl, 3-Butenyl, and 4-Methylsulfinylbutyl-GS. Right upside down: indol-3-ylmethyl, 4-Hydroxyindol-3-ylmethyl, 1-methoxyindol-3-ylmethyl GS, and 4-methoxyindol-3-ylmethyl-GS.

**Short Introduction to *Pieris* species** – The two most destructive pest species within the genus *Pieris* are *Pieris rapae* L. (small cabbage white) and *Pieris brassicae* L. (large cabbage white). Both species are native and widespread in Eurasia but have also been introduced to other continents where they have become well established. According to the datasheet published by the Centre for Agriculture and bioscience international (CABI.org), *P. rapae* is considered a pest in South America, Canada, Europe, Asia, northern countries of Africa, and Australia (figure 1.4). At the same time, *P. brassicae* is considered a pest mainly in the subtropical region of Asia and Africa and the whole European region (figure 1.4).

*P. rapae* was reported as one of the most destructive pests of Cole crops throughout the United States of America too (Kirby and Slosser 1984). According to a study in the western USA, *Pieris* species have caused about 41% of annual crucifers' losses (Shapiro 1975). It was also considered to be one of the significant defoliators of broccoli crops that led to major market losses (Vial et al. 1991; Cranshaw and Default 1985). Without pest management strategies, *P. rapae* larvae can cause complete crop loss in crucifers (Hely et al. 1982).

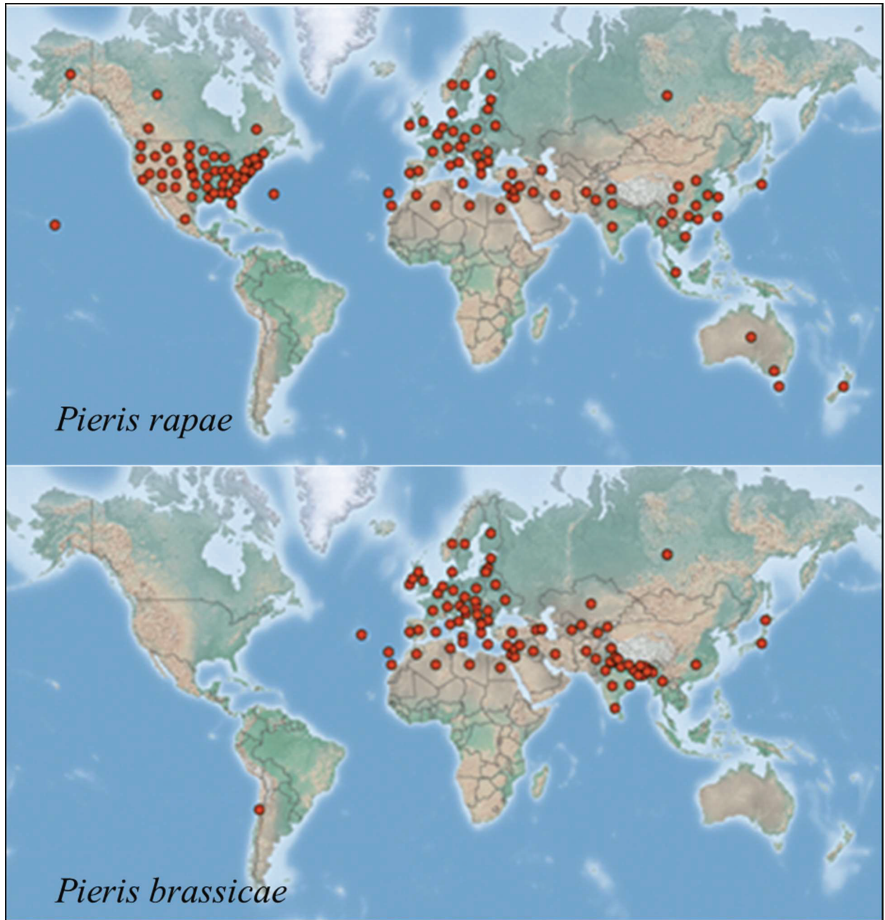
About 37 pest species of cruciferous plants have been listed from India, whereby *P. rapae* and *P. brassicae* were considered to be the most destructive ones (Lal 1975). According to a report issued in 2007, Indian agriculture has been suffered from yield losses by pests that can lead to 60-70% of yield loss (Dicke et al. 2007). *P. brassicae* is widely distributed and seen in all parts of India and Pakistan where Cole crops are widely cultivated. Larvae cause damage to all growth stages of plants (Lal and Bhajan 2004; Khan et al. 2017; Sachin and Gangwar 1980). According to a study carried out in Pakistan's northern areas, *P. brassicae* caused severe damages to all the *Brassica* cultivars used for the study. The highest population recorded was about 86 larvae per plant in the infestation peak season (Hasan and Ansari 2011; Yonus et al. 2004).

**Life Cycle of *P. rapae* and *P. brassicae*** - The life cycle of both species of cabbage butterflies has some differences, as shown in figures 1.5 and 1.6. *P. rapae* lay their eggs singly while *P. brassicae* lays 50-100 eggs in batches. *P. brassicae* shares the characteristic of laying its ova in batches with eight other British butterflies (Ford 1920; 1945). By laying single eggs, *P. rapae* has the advantage of being able to exploit isolated plants, and therefore it can be less discriminatory when choosing a suitable host for oviposition. A positive correlation between the number of eggs laid and the plant size has been recognized for *P. rapae*, predicting the minimization of larval food competition (Jones 1977, Ives 1978). *P. brassicae* is adapted for laying egg clusters in clumped vegetation and

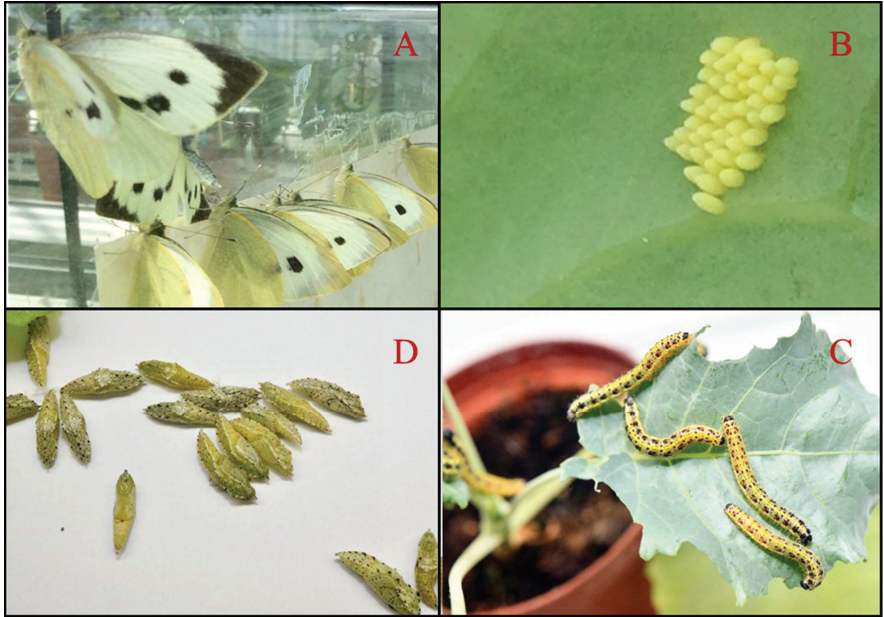
is able to maximize the utility of the host plants where larval migration does not constitute an inordinately high mortality risk (Davies and Gilbert 1985).

Both *Pieris* species select only leaves of cruciferous plants containing mustard oil glycosides on which to oviposit (Hewitt 1917). When offered cabbage and Reseda, *P. brassicae* females oviposit on cabbage first, and when the cabbage is overloaded, they switched to Reseda plants (Rothschild and Schoonhoven 1977). As the orange-yellow-colored ova mature, their color changed to a darker shade. After the larva emerges, it eats the egg case first, which contains vitamin A precursors required for growth (Fabre 1912). Both *Pieris* species have five instars and go through four larval-larval ecdyses (Klein 1932; Gardiner 1978). The larvae of *P. rapae* are velvety green with head setae partly black in color, whereas the larvae of *P. brassicae* are mottled, green with a black-colored pattern. *P. brassicae* larva is agiler than *P. rapae* over obstacles, and pupae can be found in high situations (Richards 1940). The larvae start to pupate only when they have enough materials left to make the silk girdle and cremaster (Eliot 1944). *P. brassicae* pupa is quite distinct by its large size and green, black and yellow mottling while *P. rapae* pupa is comparatively small and fresh green colored. *P. brassicae* pupae clearly show sexual differences. The female has two genital openings and is much broader than the males, as shown in figure 1.7 (Jackson 1890; Dusausoy and Delplanque 1964). Precopulation courtship in *Pieris* species occurs in the heat periods of the day (Avidov and Harpaz 1969). Copulation in *P. brassicae* commences with the male landing on or beside the female. Then the male butterfly curls its abdomen across so that it approaches the female genitalia from below, and she folded wings and making a union. Afterward, the male turns around to face away from the female (David and Gardiner 1961; Mukherji 1961). Their mating lasts from 2 to 4 hours (Chandra and Lal 1977b).

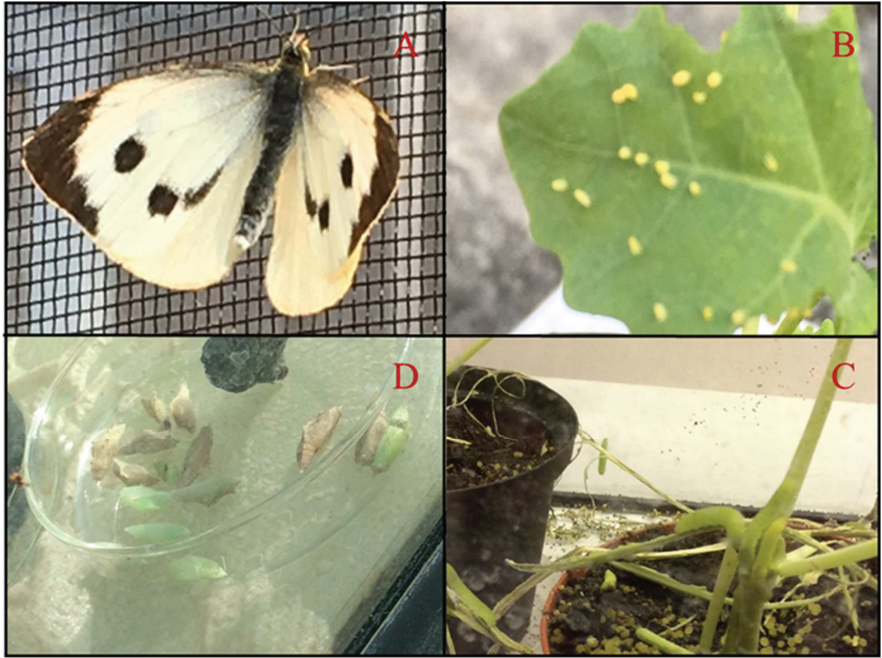




**Figure 1.4:** Maps showing the global distribution of *Pieris* species (CABI, Centre of agriculture and bioscience international; 2018)



**Figure 1.5:** Life cycle of *Pieris brassicae*. Adult butterflies (A); Eggs laid in a group (B); Third-star larvae feeding on a Turnip cabbage plant (C); Pupae (D)



**Figure 1.6:** Life cycle of *Pieris rapae*. Adult butterfly (A); Eggs laid separately on a leaf (B); Third-star larvae feeding on Turnip cabbage plants (C); Pupae (D)