INFLUENCE OF REARING TEMPERATURE ON FATTY ACID METABOLISM IN THE PEA APHID, *Acyrthosiphon pisum*

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I. Introduction

Aphids are mostly soft-bodied, plant-sucking insects, ovoid and plump in general shape, about 1-10 mm in length. They belong to the family Aphididae order Homoptera. Over four thousand species of aphids have been identified with most distributed in the temperate regions of the world (Estop, 1971). Aphids cause major economic losses in cultivated crops. They damage plants not only by removing nutrients, but also by transferring pathogens to the plants. The damage is closely associated with the aphid reproduction. Aphids build up their population very rapidly by parthenogenesis. During feeding, aphids excrete large quantities of liquid waste, called honeydew, which coats the plant surface and serves as a media for bacterial and fungal growth. Multiple aphid species attack most crop plants and some aphid species attack an incredibly broad range of plants. Therefore, many aphids are economically important pests of crops in the world.

Temperature is an important environmental factor affect aphid growth, development, behavior, and reproduction. One aphid, the greenbug (*Schizaphis graminum*) survives well all-year round and keeps a certain population density during winter in Oklahoma. How the aphids survive under cold stress is an interesting question and has practical implications for aphid control and population monitoring (Giles, personal communication).

The underlying biochemical mechanisms that are involved in the adaptation of aphids to low temperatures are not understood. Preliminary studies in our laboratory indicated that in both greenbug, *Schizaphis graminum* and the pea aphid, *Acyrthosiphon*

pisum exposure to a reduced temperature (10°C) resulted in a large increase in the storage of triglycerides. In addition, there was an increase in the amount of unsaturated fatty acids. This observation is consistent with other studies in which animals adapt to low temperature by increasing the degree of unsaturation in membrane lipids.

Lipids comprise a group of structurally heterogeneous compounds that are characterized by their solubility in solvents of low polarity. They include fatty acids, fatty acid esters of glycerol (acylglycerols) and sphingosine (sphingolipids), phosphoglycerides, waxes, terpenes, steroids, prostaglandins, lipoproteins, and glycolipids. Fatty acids have important functions in biological systems including serving as a reservoir for metabolic energy (e.g. triglycerides), as membrane structural components (e.g. phospholipids, glycolipids, and sterol esters), as precursors to compounds that regulate biochemical and physiological processes (e.g. prostaglandins), as precursors in biosynthesis of waxes, pheromones, and as components of defensive secretions (Stanley-Samuelson et al., 1988).

While feeding, aphids obtain carbohydrates and a small amount of amino acids from host plants (Dixon, 1973 & 1985). Because aphids don't get significant amounts of fatty acids from their hosts, they have to synthesize fatty acids required for life *de novo*. Fatty acid synthase plays a central role in *de novo* biosynthesis of fatty acids. In other organisms the activity of this enzyme is known to be regulated by various factors including developmental age, environmental temperature, diet, and hormones. The overall rate of fatty acid synthesis is controlled by the activity of acetyl-CoA carboxylase. The activity of this enzyme is also regulated by a number of factors. In the pea aphid, the unsaturated fatty acids all contain a Δ9-unsaturation that is most likely introduced by a

Δ9-desaturase. In other organisms Δ9-desaturase gene expression is known to be regulated by changes in environmental temperature.

Little is known about analogous hormonal control of fatty acid metabolism in insects. However, juvenile hormone and related compounds have been implicated in the control of lipid metabolism in some insects. It is believed that juvenile hormone regulates fatty acid metabolism by manipulating reproduction.

Among the aphids, the pea aphid, *Acyrthosiphon pisum* (Harris), is one of the most important pests of alfalfa, clover, pea, and other legumes. In North America, pea aphids usually feed on alfalfa, clover, pea, and faba bean. Pea aphids cause damage to alfalfa forage production and quality by removal of plant sap and transmission of plant pathogens. In addition to their importance as pests, pea aphids are relatively large aphids and are easily reared, making them good subjects for biochemical studies.

Lipid metabolism is relatively well characterized in the pea aphid, and our laboratory has extensive experience working on lipid metabolism in this insect (Dillwith et al. 1993; Neese, 1995; Brigham, 1996). Our findings from the pea aphid are expected to be applicable to other species. These studies will build a foundation for future studies on the tritrophic interactions involving the pea aphid, host plants, and predators. Understanding the molecular mechanism on fatty acid metabolism in the pea aphids may provide important insight into potential control strategies for this legume pest.

II. Objectives

The effect of low rearing temperature on fatty acid metabolism in pea aphids was investigated by completion of the following specific objectives.

I. To characterize the effects of low rearing temperature on fatty acid composition in

pea aphids. This objective was designed to test the hypothesis that pea aphids adapt to low temperature by altering their fatty acid composition.

II. To determine if precocene II blocks increased triglyceride storage at low temperature. This objective was designed to test the hypothesis that juvenile hormone mediates the accumulation of triglycerides in the pea aphid.

III. To determine the influence of rearing temperature on acetyl-CoA carboxylase, fatty acid synthase, and Δ9-desaturase activities. This objective was designed to test the hypothesis that acetyl-CoA carboxylase, fatty acid synthase, and Δ9-desaturase activities were regulated by rearing temperature in pea aphids.

IV. To isolate, clone, and sequence Δ9-desaturase cDNA fragment from the pea aphid and use it to determine Δ9-desaturase gene expression at different rearing temperatures. This objective was designed to test the hypothesis that low temperatures increase the expression of Δ9-desaturase required to produce unsaturated fatty acids for adaptation to cold temperatures.

III. Literature Review

During feeding, aphids insert their stylets into the plant tissues and suck plant phloem sap. There are carbohydrates and smaller amount of amino acids in the plant sap (Dixon, 1973; 1985). At first, aphids recognize their host plants by both physical and chemical cues emitted from the plant. After the aphids select the hosts, they choose the suitable parts (e.g. leaf or stem) and penetrate their stylets into the interstitial space between plant epidermal cells. During penetration, aphids also secrete pectinases for breakdown of connections between cells (Dixon, 1973). In the process, aphids secrete saliva which contains proteins that form a sheath around the stylets. This sheath provides

support to the stylets and enables aphids to control the direction of their stylets except at the distal end (Pollard,1973 and Dixon, 1985). When the aphids insert their stylets into the phloem, they ingest the sap by both turgor pressure of the phloem and the use of their cibarial pump (Banks, 1965 and Dixon, 1985). During feeding, aphids simultaneously inject substances into plants by the saliva that rapidly affect the plant. In the feeding area, aphids induce increased levels of amino acids and sucrose (Sandstrom et al. 2000). The physiological changes in the plant enhances the quality of the host plant facilitating rapid development and increased levels of reproduction (Neese,1995).

Aphids have two different reproduction strategies, parthenogenesis and sexual reproduction. Different morphs are produced in response to the reproductive strategy. The most common reproduction strategy in aphids is parthenogenesis, which occurs without fertilization. The offsprings produced by parthenogenesis are called viviparous. There are two different morphs, apterous (wingless) and alate (winged). Aphids increase their population rapidly by this strategy. Telescoping parthenogenesis is another reason. In telescoping parthenogenesis new embryos are developing in embryos which are developing in the viviparous aphids (Dixon, 1973). Aphids reproduce by parthenogenesis in relatively stable environmental conditions, usually throughout the spring and summer. The aphids produced by parthenogenesis are called anholocyclic.

The other reproduction strategy is sexual reproduction. Sexual reproduction is usually called cyclic parthenogenesis and is referred to as holocyclic compared to anholocyclic. The sexual forms mostly occur in autumn in response to photoperiod, temperature, and conditions of host plants. Under specific conditions, male and sexually reproducing alate females appear and mate. Fertilized eggs produced by the females

overwinter. In the next spring, first instars emerge from the herbinated eggs and after 4 molts, the nymphs develop to apterous, parthenogenic, viviparous females. All these females are called stem mothers. The offspring produced by stem mothers are similar in morphology and sex, and are referred to as virginopara. Parthenogenetic reproduction goes on during spring and summer. During this period, alates appear in response to host plant condition and population density. The alates can migrate to more suitable hosts and then continue parthenogenic reproduction.

The pea aphid

The pea aphid, *Acyrthosiphon pisum* (Harris), is long-legged, light to deep green with reddish eyes. The body length is usually 2.0-4.0 mm. Alates may be as long as 4.5 mm. Cornicles of pea aphid are characteristically long and slender. The pea aphid was first found and described in Great Britain in 1776 and distributed in North America by importing peas and clovers infested with pea aphids from Europe (Davis, 1915). Pea aphids mostly feed on legume plants, such as peas, clovers, alfalfa, and faba bean. Pea aphids extract sap from the terminal leaves and stem of the host plant. They also feed on pods causing them to curl, shrink, and partially fill. Their feeding can result in deformation, wilting, or death of the host, depending on the infestation level and size of the plant. The host plants are often coated with shiny honeydew secreted by the pea aphids, which serves as a medium for some fungi and bacteria growth. These aphids also transmit pea enation mosaic virus and yellow bean mosaic virus. The pea enation mosaic virus has been a problem in New York.

Pea aphids are distributed throughout the world and are one of the serious pests of alfalfa and some other legume plants. However, the pea aphids occur less frequently in

some Asian countries and Australia (Eastop, 1971). Pea aphids feed on susceptible alfalfa cultivars and cause economic losses (Carnahan et al., 1963). Harper and Lilly (1966), Harvey et al. (1971), Kindler et al. (1971), and Harper and Freyman (1979) reported that pea aphids also cause damage in forage production by reducing the nutrient, percent digestibility, and winter hardiness.

The developmental rate of pea aphid increases with the increasing of temperature and decreases as the upper lethal temperature (28°C) is approached. At developmental threshold temperature (5°C), the development of the aphids will pause (Lamb, 1992). The survival rate of aphids at subzero temperatures was much greater in aphids that were first adapted to 10°C than those reared at 25°C. These results suggest that adaptation at 10°C results in biochemical changes in the aphid that allows survival at subzero temperatures.

Lipids and Fatty Acids

Fatty acids are carboxylic acids with long-chain hydrocarbon side groups. The side groups are usually even carbons and are 12-20 carbons in length. The fatty acids can be divided into two groups, saturated and unsaturated. Saturated fatty acids contain no double bonds while unsaturated acids contain one to five.

In most insects, triglycerides are the primarily used for energy storage. The energy used for movement, egg development, and embryonic tissues comes from the fatty acid component of triglyceride. By β-oxidation, free fatty acids, derived from triglycerides, produce large amounts of ATP. Fatty acids are degraded by two carbons, producing one acetyl CoA, one NADH and one $FADH_2$ in one β-oxidation cycle. The acetyl CoA enters the citric acid cycle and produces 3 NADH and 1 $FADH_2$. NADH and $FADH_2$ yield ATP by oxidative phosphorylation. So fatty acids provide a major

energy source for insect activities and metabolisms.

Four major types of triglycerides are found in aphids. They are trimyristin, 2-hexano-1,3, dimyristin, 2-trans, trans,-sorbo-1,3, dimyristin, and 2-trans, trans, trans-octa-2,4,6,-trieno-1,3, dimyristin (Dillwith et al., 1993)

Temperature and Fatty Acid Metabolism

Temperature, one of the most important physical factors of the environment, affects the physiological and behavioral interactions of insects and plants (Benedict and Hatfield, 1988). At low temperature, insect development and reproduction decrease and at subzero temperature in winter, freeze tolerant insects usually enter diapause. Cold adapted insects have increased levels of ice nucleating agents, polyhydroxy alcohols, sugars, and antifreeze proteins to tolerate freezing temperatures. Meanwhile, insects usually stay under vegetation, bark of trees, rocks, or in the soil. This physical protection and biochemical agents are not sufficient to keep the insect alive. Another important metabolic change is increased levels of total fatty acids to obtain energy and increase membrane fluidity.

Researchers reported that reduced temperature could affect insect growth, behavior, and reproduction. However, little is known about how low temperature affects fatty acid storage in insects including aphids. Sutherland (1968) reported that there was an accumulation of total fatty acid in the pemphigine aphid, *Thecabius affinis*, reared at 8°C.

Many researchers (Burges, 1959; Burges, 1960; Sutherland, 1968; Karnavar and Nair, 1969; Valder et al., 1969; Lambrement and Blum, 1963; Pullin, 1987; Leather et al., 1993; Ohtsu et al., 1993) found that during diapause insects used accumulated lipid reserves as an energy source. Leather et al. (1993) noted that most diapausing insects use

lipids as their primary energy source. Lipids in the form of triglycerides are one of the most efficient storage molecules because their oxidation generates more calories per gram than either proteins or glycogen. The amount of lipids obtained for overwintering is dependent on the amount of feeding that adults can do before the onset of diapause (Pullin, 1987). For example, both *Aglais urticae* and *Inachis io* feeding less than three days have little chance of surviving during three months of diapause, and those feeding less than seven days are not likely to survive during the winter. Species which are allowed to feed for over one week can survive longer than 5 months at 4°C. These differences in survival are attributed to insufficient lipid accumulation for those insects that are only allowed to feed for seven days or less. Similar results are also obtained in pemphigine aphid, beetles, fruit flies, and *Musca autumnalis*. Sutherland (1968) reported that storage lipids increased in overwintering population in pemphigine aphid compared to summer population. Lambremont and Blum (1963) found that stored lipids increased before diapause in the cotton boll weevil, *Anthonomus grandis,* and those lipids were primarily stored as triglycerides, which mostly consisted of heptadecanoic, linoleic, linolenic, myristic, oleic, palmitic, palmitoleic, and stearic acids. Burges (1959 & 1960) described that the khapra beetle, *Trogoderma granarium* had a very unusual diapause in that most of the larvae in diapause fed intermittently and with intermittent feeding this species might even remain in diapause for up to 4 years. While in diapause, the khapra beetle accumulate approximately 2.5 times more lipids than nondiapausing larvae (Karnavar and Nair, 1969).

There are fewer studies on the effects of temperature changes on the fatty acid composition of individual tissues and organs of insects. Chippendale (1973) found that

southeastern corn borer's larvae primarily utilized triglycerides stored in the fat body for diapause. The larvae just entering diapause contained 11.2 mg fat body lipid/larvae whereas 14-day-old nondiapausing larvae contained only 4.5 mg fat body lipid/larvae. Baldus and Mutchmor (1988) also described fatty acid contents of the nerve cord and fat body of American cockroaches. They noted that fifteen fatty acids were detected in the nerve cord and 10 were significantly affected by 7 days of temperature acclimation. Seventeen fatty acids were detected in the fat body and five were significantly affected by temperature acclimation.

In insects, it is well known that overwintering individuals accumulate low molecular mass cryoprotectants, which provide colligative depression of freezing and supercooling points and also stabilize the native state of proteins or membranes to prevent denaturation as a consequence of low temperature (Lee et al., 1986; Lee, 1991; Story and Story, 1991, Ohtsu et al., 1998). Meanwhile the insects under cold stress accumulate triglycerides for energy storage (as discussed in above section). However, all these biochemical and metabolic changes are not enough for cold tolerance. Maintaining the fluidity of cellular membranes could be a mechanism to provide cold tolerance (Hochachka and Somero, 1984; Hazel, 1985). Membrane fluidity depends mainly on the ratio of saturated/unsaturated fatty acyl chains of the phospholipids. Cold stress in some insects is known to induce changes in fatty acid composition, particularly an increase in unsaturated fatty acids, which mainly occurred in phospholipids (Keith, 1966; Baldus and Mutchmor, 1988; Ohtsu et al., 1993; Ohtsu et al., 1998). Baldus and Mutchmor (1988) reported that low temperature acclimation caused a decrease in the amounts of 3 saturated fatty acids (myristic, pentadecanoic, and palmitic) and an increase in the amounts of 3

unsaturated fatty acids (linoleic, eicosadienoic, and arachidonic) in the nerve cord extraction of American cockroaches. Palmitoleic acid increased dramatically in diapausing face flies compared to nondiapausing flies (Valder et al., 1969). Ohtsu et al. (1993 & 1998) reported that phospholipids of many cold-tolerant organisms contained more unsaturated fatty acid than cold susceptible organisms, a phenomenon known to maintain membrane fluidity at low temperature. They described that the cold tolerance of fruit flies was directly related to distribution range. Among six species of *Drosophila* group, species or populations from the northern regions were usually more cold-tolerant than those from southern region in Japan. Heat tolerance was not correlated with distribution range. In the cool-temperate species, which entered deep reproductive diapause to pass the winter in response to short day length, diapausing individuals were more tolerant of both cold and heat than nondiapausing ones. They observed that there was an increase in the percentage of unsaturated fatty acids with a decrease in rearing temperature. With enhancement of cold tolerance, the percentage of monoenoic acids increased but the percentages of dienoic acids decreased and the number of double bonds in the phospholipid decreased without marked variation in the percentages of unsaturated fatty acids. Concomitantly, the percentage of fatty acids containing 16-carbon atoms increased, while that of fatty acids with 18 carbon atoms decreased. Since phosphotidylethanolamine is a dominant phospholipid in *Drosophila*, these changes probably contribute to keeping the homeoviscosity of the cellular membranes, thereby increasing cold tolerance.

Temperature changes cause many biochemical and metabolic changes in insects. The change of fatty acid content is one of the most important metabolic changes. In

some insects, both saturated and unsaturated fatty acids increased and in other insects the ratio of unsaturated/saturated fatty acids increased under cold stress. All these changes make the insects cold tolerant. From the studies, we should figure out some regularity of pest population dynamics and then provide some strategies for integrated pest management.

Juvenile Hormone and Lipid Metabolism

Juvenile hormones are secreted by the corpora allata. Wiggleworth (1935) noted first the function of the corpora allata in the hemipteran, *Rhodnius prolixus.* He discovered that secretions of corpora allata could keep the insect in its larval stage. Roller et al (1967) first determined the chemical structures of JH. Five different juvenile hormone analogs, JH-0, JH-I, JH-II, JH-III, and 4-methyl JH-I have been identified in insects so far. Among them, JH III is the most common in insects. In addition to the above function, juvenile hormones play an important role in regulation of development and reproduction, such as caste determination in the social insects, the regulation of behavior in honeybee colonies, the polyphenisms of aphids and locusts, regulation of larval and adult diapause, vitellogenin synthesis, and ovarian development (Nijhout, 1994; Gilbert et al. 2000).

Several studies showed that JH analogs might regulate lipid metabolism. In terms of the changes of lipid, three different responses were observed in insects. 1, The fatty acid content increased when the insect corpora allata were removed, whereas fatty acid content decreased when corpora allata were implanted ; 2, The fatty acid content was not changed with either removal or implanted corpora allata; and 3, The fatty acid content was decreased when the insect corpora allata were removed, and when the corpora allata

were implanted, fatty acid content was increased.

After Pfeiffer (1945) speculated at first that juvenile hormone had an effect on lipid metabolism, many researchers studied how juvenile hormone affect lipid metabolism in insects. Removal of corpora allata of insects influences lipid metabolism. When corpora allata were removed, the lipids in the fat body were increased in many insects (Downer, 1985). Contrary to these results, the amount of lipids in the fat body decreased after the corpora allata were implanted (Hill and Izatt, 1974). This is also confirmed by the application of either JH analogs or JH agonist (JH mimic). In bumblebee queens, Roseler and Roseler (1988) demonstrated that topical application of JH-I in the insects induced accumulation of lipids in fat body. A similar result was observed in juvenile mud crabs, *Rhithropanopeus harrisii* (Nates and McKenney, Jr, 2000). They found that fenoxycarb, an insect JH analog,induced reduction of fatty acid content in the crab. These data indicate that the removal of corpora allata induces accumulation of lipids in fat body in some insects, whereas implantation of corpora allata or application of JH mimic causes opposite results.

In other insects, removal of the corpora allata doesn't affect lipid metabolism. Van Handel and Lea (1970) found that removal of corpora allata (allatectomy) didn't induce changes of lipid content in the fat body compared to the control in the mosquito, *Aedes taeniorhynchus* (Wiedemann). In the cockroach, *Blaberus discodalis*, Mannix and Keeley (1980) described a similar result. Neese et al. (2000) reported that JH analogs didn't affect the fatty acid content in the American dog tick, *Dermacentor variabilis* (Say) and in *Ornithodoros parkeri* Cooley.

Contrary to the above descriptions, application of JH agonists (methoprene) and JH

antagonists (precocene II) induced different results. Application of methoprene in the larvae of the eastern spruce budworm, *Choristoneura fumiferana* (Clemens) induced accumulation of lipids in fat body (Mulye and Gordon, 1993). A similar result was obtained in pea aphids (Neese, 1995). He found that application of methoprene (JH mimic) caused accumulation of fatty acids, whereas precocene II caused reduction of fatty acid content.

The mechanisms for fatty acid changes in relation to removal or implantation of corpora allata in insects is not clearly understood yet. Beenakkers (1983) speculated that accumulation of fatty acids in response to removal of corpora allata was related to the prevention of egg ripening, increased or constantly high feeding activity, and increased lipid synthesis in fat body. Chinzei et al. (1981) described that vitellogenin contained about 8% lipid in the migratory locust, *Locusta migratoria* L. and these lipids could be stored in the fat body after prevention of egg ripening. This may not be a plausible mechanism. In terms of Hagedorn and Kunkel (1979), yolk synthesis was not directly controlled by JH in all insects. On the other hand, accumulation of lipids in fat body was also observed in male insects after removal of the corpora allata (Odhiambo, 1966; Walker and Bailey, 1971a). So, accumulation of lipids in fat body is not wholly correlated with the prevention of egg ripening.

In some insects, accumulation of fatty acids in fat body was related to increased level of feeding after allatectomy (Walker and Bailey, 1971a). They observed that the feeding of the desert locust, *Schistocerca gregaria* (Forsk.) increased after allatectomy. However, this is not the case in all insects. Odhiambo (1966), Hill and Izatt (1974) demonstrated that accumulation of lipid in fat body occurred in the allatectomized locusts and food

consumption was similar between treated and untreated locusts. In the migratory locust, feeding was not affected by either removal or implantation of corpora allata (Beenakkers and Van den Broek, 1974). These findings suggest that the mechanism of accumulation of lipids in fat body after allatectomy is not closely related to the insect feeding (Neese, 1995).

The accumulation of lipids in fat body is closely related to the increased level of lipogenesis (Neese, 1995). More [1-^{14}C] acetate was incorporated by allatectomized female cockroaches compared to control insects (Vroman et al. 1965). In the desert locust, Walker and Bailey (1971b) noted a similar result. They found higher [1-^{14}C] acetate incorporation into allatectomized locusts. All these processes should be related to a specific enzyme activity in lipogenesis. In the allatectomized male cockroaches, glucose-6-phosphate dehydrogenase activity increased (Walker and Bailey, 1971c). Keeley (1978) also described a similar result in allatectomized migratory locust. He found that the specific enzyme activity increased two to three times in lipogenesis.

Overall, in terms of the above descriptions, there are three types of reactions in insects in response to either removal or implantation of corpora allata. Studies on the pea aphid showed that JH mimic induced the accumulation of fatty acids and JH antagonist induced the reduction of fatty acid content. However, Our understanding of the effects of JH on aphid fatty acid metabolism is not complete. Understanding how JH antagonists affect fatty acid metabolism in pea aphids in response to environmental conditions will provide important information about pea aphid physiology and may provide clues for better control of aphid pests.

Acetyl CoA Carboxylase (ACCase) and Fatty Acid Metabolism

The formation of malonyl CoA is the committed step in fatty acid synthesis. Acetyl CoA carboxylase (ACCase) catalyzes the synthesis of malonyl CoA from bicarbonate and acetyl CoA. ACCase is a biotin containing enzyme that catalyzes the ATP-dependant carboxylation of acetyl CoA to yield malonyl CoA (Stryer, 1997). The active form of the enzyme is a polymer. Animal ACCase has the unique property of forming linear polymers in the presence of fatty acyl-CoA esters, malonyl CoA, or ATP plus bicarbonate which promote depolymerization (Browsey and Denton, 1987).

ACCase activity was affected by various factors in the observed organisms. The diet with high amount of unsaturated fatty acid could decrease the ACCase activity in *C. capitata* (Lizarbe, 1980). Municio et al. (1974) observed that ACCase activity was different in response to different life stages in *C. capitata*. ACCase activity increased quickly during larval stages and reached the maximum in the early 3rd instar. It declined soon and became very low during the pupal stage. Fatty acid synthesis is regulated by phosphorylation–dephosphorylation reaction. In the process, insulin stimulates the dephosphorylation of acetyl-CoA carboxylase, activating fatty acid synthesis. Wolf (1996) described that chloramphenicol acetyl transferase (CAT) activity and mRNA in lipogenetic tissues increased 10-fold in mice after injection of insulin, whereas the diabetic mice had undetectable CAT mRNA. Phosphorylation of acetyl-CoA carboxylase by the hormone epinephrine, norepinephrine, and glucagon resulted in the inactivation of the enzyme and inhibited the synthesis of fatty acids from acetyl-CoA. Neese (1995) demonstrated that ACCase activity in pea aphids reared on faba bean was significantly higher compared to those on alfalfa using either acetyl- or propionyl-CoA as substrates.

Since ACCase catalyzes the rate-determining step in fatty acid synthesis, determining

the ACCase activity in the pea aphid reared at different temperatures is of importance. In our study, we will test the hypothesis that ACCase activity is different in response to different rearing temperatures.

Fatty acid synthase (FAS) and fatty acid metabolism

Fatty acid synthase (FAS) plays an important role in the lipogenic pathway. Animal FAS consists of two identical polypeptides, each containing six enzymes and one acyl carrier protein, which together convert acetyl-CoA and malonyl-CoA to palmitate. It is dimeric with a subunit molecular weight of 250kDa. The formation of palmitic acid results from the condensation of an acetyl moiety with seven malonyl moieties. The malonyl/acetyl transferase and β-ketoacyl synthase of one subunit cooperate with the reductase, acyl carrier protein and thioesterase (TE) of the companion subunit in the formation of a center for fatty acid synthesis. Initiation of the series of condensation reactions needs the translocation of an acetyl moiety and subsequently seven malonyl moieties from CoA thioester to the 4'-phosphopantethaine of the acyl carrier protein domain of the FAS. A single transacylase enzyme is responsible for translocation of both substrates and intermediates at the same site (McCarthy et al., 1983). The substrates bind to the transacylase domain randomly and each substrate is a competitive inhibitor for the other. Both substrates bind to the enzyme in sequence and the substrate which binds inappropriately must be moved rapidly by transfer back to the CoA acceptor (Stern et al., 1982).

In lipogenesis, each condensation step increases the acyl chain by two methylene groups and consumes two NADPH (Wakil et al., 1983). The binding of NADPH triggers dimerization of FAS monomer and occurs at the enoyl reductase domain

(Polouse et al., 1981).

In pea aphid, the saturated fatty acid produced by FAS is predominantly myristic acid. This is due to involvement of an another enzyme, thioesterase II (Ryan et al., 1982; Dillwith et al., 1993; Neese, 1995; and Brigham, 1996). The enzyme cleaves the growing acyl chain at myristate (14:0) and produces shorter chain fatty acids compared to palmitate. The biosynthesis of fatty acids in organisms is related to fatty acid synthase activity.

FAS activity was affected by various factors, such as nutrition, hormones, environmental condition, and age. Xu and Rooney (1997) demonstrated that glucocorticoids led to increases of fatty acid synthase mRNA stability in fetal rat lung. In fetal lung, biosynthesis of phosphotidylcholine increased by application of glucocorticoids and this effect was mediated by increased expression of the FAS gene. They observed that the half-life of FAS mRNA was 4 hours in control explants cultured without hormone, whereas FAS mRNA stability increased 84% in the explants cultured with dexamethasone (an artificial glucocorticoids) for 44 hours and by 40% in those cultured with the hormone for 5 hours. This result indicated that glucocorticoids enhanced expression of the FAS gene in fetal lung by increasing mRNA stability and stimulating the transcription.

Nutrition and amount of diet can affect the fatty acid biosynthesis in organisms. Smith et al. (1992) recorded that increased *de novo* lipogenesis was related to graded increases in the level of food intake. When transgenic mice were starved for 48 hours and then refed a high-carbohydrate (58%), fat-free diet for 24 hours, chloramphenicol acetyl transferase (CAT) activity was increased fivefold (Wolf, 1996). Goodridge et

al. (1989) also demonstrated that short-term fasting reduced lipogenesis in chicken, whereas meal size increased the propotion of glycogen synthesized by rats (Obeid et al. 1998). A diet with a high calorie: protein ratio led to an increased level of lipogenesis (Donaldson, 1985). Rosebrough et al. (1986) and Rosebrough et al. (1987) observed similar results. Low protein (12%) diets have been shown to elevate lipogenesis compared to high protein (30%) diets. Intake of fat, especially polyunsaturated fatty acids led to decrease of fatty acid synthase activity. Moibi (2000) reported that dietary lipid supplementation reduced FAS activity by 30% in all three fat depots in sheep. In mice, FAS activity was not affected after they were fed with 10% triolein (monounsaturated fatty acid) whereas it decreased about 2-fold after intake of 10% menhaden oil (polyunsaturated fatty acids) (Wolf, 1996). Therefore, the level of food intake and nutritional level have a profound impact on the rate of fatty acid biosynthesis. Researchers confirmed that host plants could affect fatty acid content in insects. Neese (1995) observed that pea aphids reared on alfalfa had a 40% higher triglyceride content those reared on faba bean. But this result did not relate to FAS activity. FAS activity in pea aphids reared on alfalfa was not significantly different that in pea aphids reared on faba bean (Neese, 1995).

Moibi et al. (2000) demonstrated that temperature and dietary lipids could affect enzyme activity with differential response in different tissues. FAS activity was not affected by environmental temperature and dietary lipid in longissimus dorsi muscle and liver. FAS activity was reduced in subcutaneous adipose tissue in response to cold stress, but increased FAS activity was observed in mesenteric adipose tissue. Similar results were reported by Nicholls and Locke (1983) and Buckley and Rath (1987).

In insects, however, there were no studies on fatty acid synthase activity in response to different rearing temperature. The preliminary results of our study in the pea aphid showed that fatty acid level especially myristic acid was much higher in 10°C aphids compared to that of 25°C aphids. Fatty acid synthase activity might increase in the pea aphids under cold acclimation. One objective of our study was to determine fatty acid synthase activity in pea aphids in response to different rearing temperature.

Δ9-desaturase activity and fatty acid metabolism

Any organism has the potential ability to modify fatty acid composition of their membrane phospholipids in response to changes in environmental temperature (Marcartney et al., 1994). Such compositional adaptation of membrane lipids is usually called a homeoviscous adaptation process. It maintains the proper membrane fluidity during cold stress (Sinensky, 1974; Nakashima, et al., 1996). Δ9-desaturase, which puts the first double bond between C9 and C10 position of long chain saturated fatty acids plays an important role in the process. This oxidative reaction is catalyzed by the iron-containing, microsomal enzyme, and involves cytochrome b_5, NADH or NADPH-cytochrome b_5 reductase, and molecular oxygen. In the reaction, the preferred substrates are palmitoyl- and stearoyl-CoA, which are converted into palmitoleoyl- and oleoyl-CoA, respectively. Δ9-desaturase generally contains 300-500 amino acid residues and has two potential double membrane spanning regions and three conserved histidine cluster motifs (one HXXXXH and two HXXHH) (Shanklin et al., 1994).

Δ9-desaturase activity was affected by various factors in the observed organisms. Its activity was sensitive to dietary changes, hormonal imbalance, developmental processes, temperature changes, metals, alcohol, peroxisomal proliferators, and phenolic compounds

(Ntambi, 1999). The ratio of saturated to unsaturated fatty acids changed abnormally in mammals suffering several diseases such as diabetes, cardiovascular disease, obesity, hypertension, neurological disease, immune disorders, cancer, and aging (Kinsella et al., 1990; Jones et al., 1996; Pan et al., 1994; MacGee, 1981; Garbay et al., 1998). Therefore the regulation of Δ-9 desaturase has a considerable physiological importance.

In male houseflies, desaturase activity increased about 26-fold from emergence until the insects were three and four days old. In females, the activity increased about 12-fold from emergence until day 2, then it sharply decreased on day three and maintained 6-8-fold increase for the next four to five days. After 11 days, the activity had decreased to the value observed at emergence in both sexes (Wang, et al., 1982). A similar result was reported by Cripps et al. (1988) in cricket. In carp, researchers (Tiku et al., 1996; Trueman et al., 2000) demonstrated that Δ9-desaturase activity increased in response to cold acclimation. While carp were progressively cooled from 30 to 23, 17, and 10°C, the saturated/unsaturated fatty acid ratio decreased linearly. They observed that Δ9-desaturase activity increased 2.5-fold at 23°C on day one and there were no changes in the amount of desaturase protein or transcript. At 17°C, desaturase activity was greatly increased and reached 7-fold on day five and desaturase transcription increased 10 to 20-fold. At 10°C transcription of desaturase gene increased 40 to 50-fold, however desaturase activity did not increase compared to that at 17°C.

Cheesbrough (1990) described a similar result in soybean. Soybean seeds that were grown at 20°C showed an increase in stearoyl-acyl carrier protein desaturase activity whereas the seeds that were grown at 35°C showed decreased enzyme activity.

Batcabe et al. (2000) noted that Δ12-desaturase activity in cricket was also affected

by developmental age, envioronmental temperature, and diet. Δ12-desaturase activity gradually increased in ultimate instars from 2.2 to 5.5% at 3-4 days post-eclosion. In adult cricket, the desaturase activity increased to 13.3% at day four post-eclosion. When the insects were reared at 17 and 15°C from room temperature for 24 and 48 hours the Δ12-desaturase activity was not significantly different. When the cricket was reared at 13°C for 72 hours, Δ12-desaturase activity was 66% higher than that of the insects reared at room temperature. In both adults and ultimate instars, Δ12-desaturase activity decreased 60% in relation to starvation.

Overall, desaturase activity is affected by various factors, such as developmental age, environmental temperature, diet, and hormones, similar to the fatty acid synthase. Although the desaturase activity was observed in several organisms, it was not studied in aphids yet in response to various factors. The objective of the study was to characterize Δ9-desaturase activity at different rearing temperatures.

Expression of Δ9-desaturase genes in response to different temperatures

Among the acyl-CoA desaturases, genes encoding Δ9-desaturase homolgs have been cloned and sequenced in different organisms, including fungi, bacteria, yeasts, protozoa, arthropods, and vertebrates. In arthropods, the genes of tick (Luo et al., 1997), house cricket (Riddervold et al.*), fruit fly (Wicker-Thomas et al., 1997), the cabbage looper moth (Liu et al., 1999), redbanded leafroller (Liu et al.*), European corn borer (Liu et al.*), corn earworm (Rosenfield and Knipple*), and silkworm (Yoshiga et al., 2000) had been cloned and sequenced and finally the amino acid sequences were deduced. They found that these desaturases, containing 300-500 amino acid residues,

* From http://www.ncbi.nlm.nih.gov/

had two potential double-membrane-spanning regions and three conserved histidine cluster motifs, one HXXXXH and two HXXHH (Shanklin et al., 1994). Histidine clusters were bound to iron atoms and played an important role in the introduction of double bonds into saturated fatty acids (Murata and Wada, 1995). In Δ9-desaturases, arginine and proline residues were also highly conserved. Arginine residues were involved in the binding of the negatively charged CoA moiety of the substrate (Ercoch and Strittmatter, 1978).

Expression of desaturase mRNA was examined by Northern blot analysis in carp (Tiku et al., 1996) and in a protozoan, *Tetrahymena thermophila* (Nakashima et al., 1996) at different temperatures. They described that increases in gene expression in response to cold temperature were due, at least in part, to transcriptional activation. In *Tetrahymena thermophila,* transcription of the desaturase gene was increased 2.0-fold at 23°C and 2.6-fold at 15°C compared to that of 35°C (Nakashima et al., 1996). Cold induced Δ9-desaturase expression in carp was also related to posttraslational effects (Tiku et al., 1996).They noted that a preexisting latent desaturase became activated 24 to 48 hours after initiation of cooling. The release of sequestered enzyme or activation by posttranslational modification was involved in the process. This effect might be an early response to moderate cooling, whereas transcriptional up-regulation and increased desaturase protein might require much lower temperature. The elevation of the desaturase mRNA was also related to its stability (Nakashima et al., 1996; Tiku et al., 1996). Nakashima et al. described that the half-life of the desaturase mRNA in cells growing at 35°C was less than 45min, whereas at 15°C it was more than 60min. This result indicated

that increased level of the desaturase mRNA at cold temperature was partly due to its increased stability.

Amount of Δ9-desaturase mRNA increased with the increasing of the cooling time. Tiku et al. (1996) demonstrated that the amount of Δ9-desaturase mRNA was low in warm acclimated carp and after 24 hours of cooling. However, it increased greatly after 48 hours cooling. After 3 to 5 days it reached the highest level. Then it decreased gradually over time and maintained a relatively high level. A similar result was reported in *T. thermophila* by Nakashima et al. (1996). However, we do not know how temperature regulates the gene expression in all observed organisms. Ntambi (1999) demonstrated that the expression of the mouse stearoyl-CoA desaturase gene was regulated by polyunsaturated fatty acids and cholesterol at the levels of transcription and mRNA stability.

Two mouse and rat Δ9-desaturase genes (SCD1and SCD2) were cloned and sequenced. Ntambi (1999) described that their expression was affected by both polyunsaturated fatty acids and cholesterol. Pascal et al. (2001) observed a similar result in the chicken. Ntambi (1999) demonstrated that a certain region of the promoter of the two mouse genes differed markedly, however, there was one region with high nucleotide sequence homology. The sequence between –201 to –54 in the SCD2 gene was 77% identical to the sequence between –472 to –325 in the SCD1 gene. The regulatory elements responsible for polyunsaturated fatty acids and cholesterol regulation of the gene were contained within the conserved region (Ntambi, 1999; Pascal et al., 2001). In mouse, polyunsaturated fatty acid and cholesterol repressed the maturation of the sterol regulatory element binding protein (SREBP) by the SREBP cleavage-activating protein (SCAP). SCAP usually translocated from the endoplasmic reticulum (ER) into the nucleus to activate the transcription of the SCD genes by binding to the sterol regulatory

element (SRE). Polyunsaturated fatty acids either from the diet or membrane phospholipid (mPL) independently bind the putative binding protein (PUFA-BP) and the complex of PUFA and PUFA-BP represses transcription of the Δ9-desaturase genes by binding to the polyunsaturated fatty acid response element (PUFA-RE) (Ntambi, 1999). In chicken, Pascal et al. (2001) observed that the presence of a 5'AGCAGATTGCG 3' element at position –365 to –355, which is similar to that described in mouse SCD1 and SCD2 genes and shown to be a novel, functional sterol regulatory element (SRE). A CAAT-like, NF-Y (nuclear factor Y binding site) element was also observed at position –349 to –345, 5 bp downstream of the novel SRE element. The composite NF-F and SRE motif was similar to that observed in rodent SCD1 and SCD2 promoters. Pascal et al. (2001) also observed another element which corresponded to an overlapping Sp1 and USF motif at –180 to –158, which played a role in insulin/glucose and PUFA regulation (Pascal et al., 2001).

Compared to rodent SCD gene regulation by PUFA, the regulation of SCD gene in chicken did not involve the peroxisome proliferator-activated receptor (PPAR) pathway, since the clofibrate peroxisome proliferator had no effect on chicken SCD gene expression (Pascal et al., 2001). The regulation of the gene expression by PUFA was similar to that of mouse.

Although Δ9-desaturase genes have been cloned and sequenced in several insect species so far, the gene expressions in response to different rearing temperatures are not conducted yet in insects. From our temperature study in the pea aphids, we have found that unsaturated fatty acids increase also in response to low rearing temperatures. We assume that this increase may relate to increased level of Δ9-desadurase gene expression.

The objective of our study is to determine how unsaturated fatty acid metabolism in the pea aphid responses to different rearing temperatures.

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Influence of Rearing Temperature on Triglyceride Storage in the Pea Aphid, *Acyrthosiphon pisum*

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Abstract

Pea aphids reared at 10°C (3.14 ± 0.17 mg) was significantly heavier than aphids reared at 25°C (2.11 ± 0.22 mg). The aphid weights at 5°C and 4°C under sequential decrease of rearing temperature (treatment 2) were significantly heavier than those in the sudden decrease of rearing temperatures (treatment 1) (3.02 ± 0.20 mg vs 2.42 ± 0.10 mg at 5°C and 2.96 ± 0.14 mg vs 2.02 ± 0.14 mg at 4°C).

Total fatty acid including saturated fatty acid and unsaturated fatty acid in the pea aphid reared at low temperatures increased significantly compared to that in high rearing temperature. This change is mostly reflected in the large increase of myristic acid, which occurs exclusively in triglycerides. When aphids were moved from 25°C to a lower rearing temperature, saturated fatty acids accumulated over a period of time, reaching a maximum at 16 days after initial exposure to low temperature. Aphids moved to 4°C, a temperature below the developmental threshold, did not accumulate saturated fatty acids. Similar results were observed when aphids were exposed to sequential decrease in rearing temperature.

Acetyl-CoA carboxylase activity in the aphids at 25°C was 2-fold higher than that in the aphids at 10°C. Fatty acid synthase activities in the aphids reared at 25°C and 10°C are quite similar (96.08 ± 8.50 vs 97.76 ± 3.76; mean± standard deviation; unit: nmoles NADPH/min/mg protein). These data suggest that increased levels of fatty acid biosynthesis is not responsible for accumulation of saturated fatty acids.

Aphids reared at 10°C showed a 3-fold reduction in reproduction rates. This reduced production of new nymphs reduces energy demand and would allow for accumulation of energy store (triglycerides). Therefore, the increased level of saturated fatty acids in aphids reared at low temperature is probably related to lower utilization of fatty acids rather than increased rates of biosynthesis.

1. Introduction

Temperature is one of the most important physical factors of the environment, affecting the physiological and behavioral interactions of insects and plants (Benedict and Hatfield, 1988). At low temperatures, insect development and reproduction decrease and at subzero temperatures, freeze tolerant insects usually enter diapause. The developmental rate of pea aphids, for example, increases as rearing temperature increases and then decreases as the upper lethal temperature (28°C) is approached. At the developmental threshold temperature (5°C), the development of the aphids will pause (Lamb, 1992). The survivorship of aphids at subzero temperatures was much greater in aphids that were first adapted to 10°C than those reared at 25°C. Cold adapted insects have increased levels of ice nucleating agents, polyhydroxy alcohols, sugars, and antifreeze proteins to tolerate freezing temperatures (Bale, 1993). In addition, insects usually stay under vegetation, bark of trees, rocks, or in the soil. This physical protection and biochemical agents, however, are not sufficient to keep the insect alive. Insects must also rely on another important metabolic change, that of increased levels of fatty acids to provide energy and unsaturated fatty acids to increase membrane fluidity (Leather et al., 1993).

During diapause, insects use accumulated lipid reserves as an energy source. For example, storage lipids increase in overwintering populations of pemphigine aphids compared to summer populations (Sutherland, 1968). The cotton boll weevil, *Anthonomus grandis* also has increased levels of lipids before diapause that are primarily

stored as triglycerides (Lambremont and Blum, 1964). The diapausing khapra beetle accumulates approximately 2.5 times more lipids than nondiapausing larvae (Karnavar and Nair,1969).

Although much is known about the effects of diapause on fatty acid composition, there are fewer studies on the effects of temperature changes on the fatty acid composition of individual tissues and organs of insects. Chippendale (1973) found that southwestern corn borer larvae primarily utilized triglycerides stored in the fat body for diapause. The larvae entering diapause contained 11.2 mg fat body lipid/larvae whereas 14-day-old nondiapausing larvae contained only 4.5 mg fat body lipid/larvae. Baldus and Mutchmor (1988) described fifteen fatty acids in the nerve cord of the American cockroach, *Periplaneta americana*, 10 of which were increased significantly after 7 days of temperature acclimation. Seventeen fatty acids were detected in the fat body of the insect and 5 were increased significantly by temperature acclimation.

Studies have shown that increased levels of fatty acids in response to different conditions is related to fatty acid biosynthesis. Two enzymes, acetyl CoA carboxylase (ACCase) and fatty acid synthase (FAS) play an important role in the biochemical process.

ACCase activity can be affected by various factors. Diets with high amounts of unsaturated fatty acid were shown to decrease the ACCase activity in *C. capitata* (Lizarbe, 1980). ACCase activity increased quickly during larval stages and reached its maximum in the early 3rd instar of *C. capitata*. It declined soon thereafter and was very low during the pupal stage (Municio et al., 1974). ACCase activity in pea aphids reared on faba bean was significantly higher compared to those on alfalfa using either acetyl- or

propionyl-CoA as substrates (Neese, 1995).

Fatty acid synthase (FAS) plays an important role in lipogenic pathway. Animal FAS consists of two identical polypeptides, each containing six enzymatic activities and one acyl carrier protein, which together convert acetyl-CoA and malonyl-CoA to palmitate. It is dimeric with a subunit molecular weight of 250 kDa. In the pea aphid, the saturated fatty acid produced by FAS is predominantly myristic acid. This is due to involvement of an another enzyme, thioesterase II (Ryan et al., 1982; Dillwith et al., 1993; Neese, 1995; and David, 1996). The enzyme cleaves the growing acyl chain at myristate (14:0) and produces shorter chain fatty acids compared to palmitate. The biosynthesis of fatty acids in organisms is related to fatty acid synthase activity.

FAS activity is affected by various factors, such as nutrition, hormones, environmental conditions, and age in rat, mice, chicken, sheep, and pea aphid (Xu and Rooney,1997; Smith et al.,1992; Wolf, 1996; Goodridge et al., 1989; Obeid et al. 1998; Donaldson, 1985; Rosebrough et al.,1986; Rosebrough et al. 1987; Moibi, 2000).

Although studies have been done on the effects of temperature on fatty acid metabolism in several species of insects, little is known about the effect of rearing temperature on fatty acid content, ACCase, and FAS activities in the pea aphid. The objective of the present study was to characterize the effects of low rearing temperature on fatty acid composition, ACCase and FAS activities in the pea aphid. Our studies in the pea aphid will not only provide basic biochemical information about how aphids survive under cold stress, but also build a foundation for future studies on the tritrophic interactions involving the pea aphid, host plants, and predators.

2. Materials and Methods

2.1. Aphid colonies

Pea aphids, *Acyrthosiphon pisum* (Harris) were reared on healthy faba bean (Vicia faba L.) cv. 'Windsor', maintained at 25°C with a photoperiod of 16:8. A second colony was maintained at 10°C using the same plants and photoperiod. In the 1st treatment, pea aphids from the 25°C colony were infested on faba bean plants acclimated at 20°C, 15°C, 10°C, 5°C and 4°C, respectively. In the 2nd treatment, the aphids were infested at the same temperatures as first treatment, however, the temperature was decreased gradually and the aphids were infested from the aphids reared at higher temperature (such as 25→ 20→ 15→10→ 5→ 4°C). Aphids were transferred to the lower temperature after 16 days. In both treatments, aphids were sampled every 2 days (10 aphids/sample, 3 samples). Apterous healthy aphids were sampled in each experiment. The samples were weighed and then stored frozen at –20°C until fatty acid analysis.

2.2. Ovariole number

The aphids were placed on a carrier glass slide under a dissecting microscope. A dissecting probe was put on the thorax and then rolled backward until the internal organs were squeezed from the anus and gonopore. One or two drops of water were added onto the slide. The alimentary canal was separated first, allowing the ovarioles to float in the water. Ovariole numbers were counted under a dissecting microscope.

2.3. Reproduction rate

The virgin adult aphids were obtained by rearing nymphs having the same size (same age) from the 25°C colony. Then the aphids were reared at 25°C and 10°C in 3 replicates with 10 aphids each. The nymphs were counted every 2 days from the aphids reared at 25°C and every 5 days from the aphids reared at 10°C until the aphids stopped

reproduction.

2.4. Lipid extraction

Aphid samples from each treatment were homogenized by hand with glass/glass homogenizers in 3.8 ml of methanol: chloroform: water(2:1:0.8, v/v) solution and the lipids were extracted according to Bligh and Dyer (1959). Fifty µl heptadecanoic fatty acid methyl ester (17:0, 1 µg/µl of chloroform) was added to each sample as an internal standard. Lipid extracts were stored in chloroform containing 0.5% (w/v) butylated hydroxytoluene (BHT) at -20°C.

2.5. Separation of lipids by thin layer chromatography (TLC)

The total lipid extract was separated into lipid classes by TLC on normal phase silica gel plates (20×20 cm in size and 250 microns in thickness, Analtech, Newark Delaware). Plates were pre-washed in hexane: ether: acetic acid (70:30:1, v/v) in a TLC tank lined with filter paper to saturate the atmosphere with solvent. The plate was dried and spotted with sample and standards including a triglyceride standard (Sigma 178-3) containing mono-, di-, and tripalmitin, and a lipid standard (Sigma 178-1) containing cholesterol, cholesteryl oleate, oleic acid, oleic acid methyl ester and triolein. The concentration of standards was 8.325 mg/ml for the triglyceride and 10 mg/ml for the lipid standard dissolved in chloroform. One µl of each standard and 100 µl of each sample were applied to the TLC plate. The plate was developed in hexane: ether: acetic acid (70:30:1, v/v) and the phospholipids were scraped off and placed in 15 ml glass centrifuge tube for extraction. The plate was first viewed under UV (254 nm) and the area quenching fluorescence corresponding to sorbic acid containing triglyceride was marked. The plate was then placed in a TLC tank containing iodine crystals and allowed to

develop for 30-40 min. The lipid spots were marked and the iodine allowed to vaporize. Lipid classes were identified by comparison to standards and were scraped off and placed in 1 ml glass centrifuge tubes. One hundred μl of internal standard, 17:0 methyl ester (1 μg/μl) was added to each fraction. Lipids, except phospholipids, were then extracted three times with 2 ml of ether. The extract was dried under nitrogen and fatty acid content determined as described below.

Phospholipids were extracted after addition of 100 μl of 17:0 methyl ester (1 μg/μl) as an internal standard. Three ml of chloroform:methanol:acetic acid:water (50:39:1:10, v/v) were added to the tube, vortexed, and then centrifuged at low speed. The supernatant was removed and the gel extracted again with the same solvent. The supernatant was combined and 4 M NH_4OH (1 ml/ 3 ml solvent) was added. The tube was vortexed, centrifuged as above, and the chloroform phase was removed. One ml of chloroform was added, and the mixture was vortexed, centrifuged as above, and the chloroform layer was removed and combined with the first. The solvent was dried under nitrogen and fatty acid content was determined as described below.

2.6. Fatty acid analysis

Methyl esters from the lipids from total extracts or TLC fractions were analyzed based on the method of Shipley et al. (1993). Fatty acid methyl esters (FAMES) were analyzed by gas chromatography (GC) on a HP5890 Series II gas chromatograph with HP7673 autosampler (Hewlett Packard, Santa Clarita, CA). FAMEs were introduced onto a DB-225 column 30 m x 0.25 mm with a 0.15 μm film thickness (J&W Scientific, Folsom, CA) using a split injector set at 250°C with a 1:10 split ratio. Ultrapure helium was the carrier gas at 1ml/min and the GC program was as follows: 120°C for 2 min,

10°C/min to 200°C, 5°C/min to 225°C. A flame ionization detector, operating at 300°C, was used and peak areas were recorded by HP-Chemstation software. FAME peaks were identified by comparison of retention times with authentic FAMEs (NuCheck Prep, Inc., Elysian, MN).

2.7. Acetyl-CoA carboxylase(ACCase) activity assay

ACCase activity was determined in the aphids reared at two temperatures, 25°C and 10°C. Isolation of acetyl-CoA carboxylase was based on the method of Rainwater and Kolattukudy (1982). The enzyme activity of the crude homogenate was measured by combining the following: 200 µl of the 105,000 g supernatant with 10 µl of ATP (0.3 µmole/5 µl); 20 µl of acetyl CoA (0.1 µmole/10 µl); 20 µl of $NaHCO_3$ [10 mM, including 0.0178 mM radiolabeled (specific activity: 56 mCi/mmol) and 9.9822 mM unlabeled sodium bicarbanate]; and 200 µl of reaction buffer. The reaction buffer contains 50 mM Tris-acetate, pH 8.0; 20 mM potassium citrate; 10 mM magnesium chloride; 150 µg /100 µl bovine serum albumin. The mixture was incubated at 30°C for one hour. The reaction was stopped by adding 100 µl of 6 M HCl and drying under N_2. One ml of water and 10ml of Econo-Scint™ (Fisher Scientific, Houston, TX) were added to the vial and mixed. The amount of ^{14}C incorporated into malonyl CoA was determined by liquid scintillation counting.

2.8. Fatty acid synthase (FAS)

Fatty acid synthase activity was determined in aphids reared at both 10°C and 25°C.

2.8.1. Fatty acid synthase preparation

Pea Aphids were reared at different temperatures as described above. Insects were collected and immediately homogenized with a Tekmar Tissumizer (Tekmar Co.) in 50

mM potassium phosphate buffer, pH 7.4, containing 1 mM ethylene diamine tetra-acetic acid (EDTA), 1 mM ethylene glycol tetra acetic acid (EGTA) and 1mM dithiothreitol (DTT), (Brigham, 1996). All operations were carried out on ice or at 4°C. The number of aphids required for each experiment was determined experimentally. Approximately 10 ml of buffer was used per gram of aphids. The homogenate was centrifuged at 500 g for 5 minutes and the supernatant collected and centrifuged at 10,000 g for 20 minutes. The supernatant was filtered through a thin layer of glass wool to remove caked lipid. The filtrate was then centrifuged at 105,000 g for one hour. The supernatant was used for fatty acid synthase activity determinations.

2.8.2. Fatty acid synthase assay

Fatty acid synthase activity was determined spectrophotometrically by monitoring the change in absorbance at 340 nm (Ryan et al., 1982) as modified by Brigham (1996). Protein content of enzyme preparations was determined by the method of Bradford (1976) with bovine serum albumin as the standard.

2.8.3. Determination of fatty acid synthase molecular weight

In order to determine the effect of temperature on fatty acid synthase activity, a 100,000 g supernatant was obtained from aphids reared at 25°C. This extract was fractionated on a gel filtration column run at 25°C and 10°C. The samples were run on a Sepharose 6B-CL column (90 × 1.5 cm) in potassium phosphate buffer, pH7.4, at 0.5 ml/min. Effluent was monitored at 280 nm and fractions (2 ml) were collected. FAS activity from each fraction was measured spectrophotometrically as described above. Molecular weights of eluted peaks were determined by comparison to known gel filtration standards.

2.9. Analysis of data

The statistical analysis of data was carried out using the Student's *t* Test.

3. Results

3.1. Aphid weight

Individual pea aphids reared at 10°C (3.14 ± 0.17 mg) were significantly heavier than pea aphids reared at 25°C (2.11 ± 0.22 mg). The aphid weight at 20°C (2.00 ± 0.16 mg) was similar to that of aphids at 25°C. The weights of aphids at both 15°C and 10°C in both treatments were increased significantly. The aphid weight at 5°C (2.42 ± 0.10 mg) in treatment 1 increased slightly compared to that of control, whereas it (3.02 ± 0.20 mg) increased significantly at 5°C in the treatment 2. The weight of aphids (2.02 ± 0.14 mg) at 4°C did not change in treatment 1 and increased significantly (2.96 ± 0.14 mg) in treatment 2 as compared to 25°C aphids.

3.2. Aphid ovariole number

The ovariole number of pea aphids reared at 10°C (11.40 ± 1.43) was significantly less as compared to those reared at 25°C (12.80 ± 1.21).

3.3. Reproduction rate

Aphids reared at 25°C produced about 102 nymphs during its life of 30 days, whereas the aphid reared at 10°C produced about 60 nymphs in 50 days (Fig. 1). The reproduction rate of the aphids from 25°C was 3-fold higher than that of the aphids from 10°C (Fig. 2).

3.4. The fatty acid content of pea aphids reared at different temperatures

3.4.1. The fatty acid content of pea aphids from 2 colonies

The total fatty acid content of 10°C colony aphids was about two fold higher than

that of 25°C colony aphids (Fig. 3). The increase was due in large part to increase in lauric (12:0) and myristic (14:0) acids (Fig. 4). When aphids were transferred from the 25°C colony to 10°C, their total fatty acid content increased over time reaching a maximum level around 16 days (Fig. 5).

Five lipid classes including cholesterol ester, triglyceride I, triglyceride II, triglyceride III, and phospholipids were observed by thin layer chromatography. Triglycerides were classified according to the fatty acids at C2 position (Bowie and Cameron, 1965; Febyay et al., 1992; Dillwith et al., 1993). The distribution of individual fatty acids in the 5 lipid classes from aphids reared at 25°C and 10°C is shown in table 1. Myristic acid and lauric acid occurred predominantly in the triglyceride II fraction. The unsaturated fatty acids occurred in the phospholipid fraction, as expected, with higher levels of 18:1, 18:2, and 18:3 in aphids reared at 10°C.

3.4.2. The fatty acid content of pea aphids at 2 treatments

When aphids were transferred from 25°C to lower temperatures and then sampled after 16 days, the total fatty acid content of aphids increased at 15°C, 10°C, and 5°C. It increased about 2-fold at both 15°C and 10°C and did not change at 4°C (Fig. 6). Similar results were observed when aphids were sequentially moved to lower temperatures (treatment 2) (Fig. 7). The total fatty acid content of the pea aphids reared at 5°C and 4°C, however, was higher compared to that of the aphids reared at these temperatures in treatment 1 (Figs. 6& 7). The total fatty acid in the aphids reared at 20°C increased slightly over time and then decreased to original levels after 6 days. Total fatty acids in the aphids reared at 5°C increased gradually and were significantly higher than that in the control (25°C aphids). When the aphids were reared at 4°C in treatment 1, the total fatty

acid did not change over time (Table 2). The aphids stored significantly higher amounts of fatty acids for a long period of time at 5°C and 4°C in treatment 2.

3.5. Acetyl-CoA carboxylase activity

Acetyl-CoA carboxylase catalyzes the rate limiting reaction in the fatty acid biothynthesis. Malonyl-CoA, a substrate for fatty acid biosynthesis forms from acetyl-CoA and HCO_3^- in the reaction. Accumulation of $^{14}CO_2$ into malonyl-CoA was monitored over one hour period. Acetyl-CoA carboxylase specific activity in the pea aphids reared at 25°C was significantly higher than that in the aphids reared at 10°C (Fig. 8). This result suggests that fatty acid levels in the pea aphids reared at 10°C is not due to increased biosynthesis activity.

3.6. Fatty acid synthase activity

Fatty acid synthase catalyzes a reaction from which long chain saturated fatty acid are formed. The fatty acid synthase activities (96.08 ± 8.50 vs 97.76 ± 3.76; mean ± standard deviation; unit: nmoles NADPH/min/mg protein) were similar between the aphids reared at 25°C and 10°C (Fig. 9).

3.7. Determination of molecular weight of fatty acid synthase

The FAS molecular weight was 500 kDa when measured at 25°C and 10°C (Fig. 10). Earlier studies by Brigham (1996) showed that at 4°C fatty acid synthase dissociates into the inactive monomer (250 kDa).

4. Discussion

The pea aphid, *A. pisum*, has an ability to accumulate fatty acids under cold stress, involving saturated and unsaturated fatty acids. The increased fatty acid content is primarily in saturated fatty acids associated with the triglyceride fraction. Triglycerides

were classified into four classes, trimyristin, hexanoyl-triglycerides, sorboyl-triglycerides, and 2-trans, trans, trans-octa-2,4,6-trieno-dimyristin, according to the fatty acid at C2 position of the glycerol (Bowie and Cameron, 1965; Febvay et al., 1992; Dillwith et al., 1993).

Sorbic acid, hexanoyl acid, and octa- trieno-dimyristin were not detected from any of our experiments. This is probably due to a small amount and high volatility of the short-chain fatty acids. Diglycerides and monoglycerides were also not detected from TLC although they were detected in very small amount in some aphids (Febvay et al., 1992; Itoyama et al., 2000). Total fatty acids in the pea aphids reared at 10°C increased about 2-fold compared to that of the aphids at 25°C. The changes are exclusively reflected in the changes of myristic acid. It is unusual to have myristic acid as the predominant fatty acids in organisms, including insects (Dillwith et al., 1993). A special enzyme, thioesterase II, which cleaves the growing long chain fatty acid at 14-carbon, is involved in the fatty acid biosynthesis reaction.

A plausible mechanism of the biochemical process in the pea aphids under cold stress is due to reduced turnover (utilization) compared to the aphids reared at 25°C. Pea aphids reared at 25°C show maximal rates of development (20%/day) while those at 10°C show greatly reduced rates (5.8%/day) (Lamb, 1992). At low temperature, the aphid reproduction rate is decreased significantly (3-fold). The ovariole number of the aphid reared at 10°C was significantly reduced and the difference between the aphids reared at 25°C and 10°C was 1.4. This difference may be significant for aphids population (Giles, personal communication). The energy used for development and reproduction at relatively high rates is stored as triglycerides in the aphids. The studies of acetyl-CoA

carboxylase (ACCase) and fatty acid synthase (FAS) activities in the pea aphids reared at 25°C and 10°C also support the above conclusion. The cytosolic FAS activities were quite similar at 2 different temperatures. ACCase activity in the aphids reared at 25°C is significantly greater than that in the aphids reared at 10°C. Therefore, the increased level of triglycerides at low temperature does not appear to be due to an increase level of fatty acid biosynthesis. This result is also consistent with the findings in the pea aphids reared on different hosts (Neese, 1995, PhD, dissertation).

Pea aphids survive very well under subzero temperature when they were adapted first at 10°C. The aphids accumulated some antifreeze agents, such as ice nucleating agents, polyhydroxy alcohols, sugars, and antifreeze proteins in the process (Bale, 1993). Our results from the two treatments were consistent with the statement. When the aphids were reared below the threshold temperature (4°C) from the aphids reared at 25°C, the fatty acid content did not change (treatment 1). However, in the aphids reared at threshold temperature from the aphids reared at sequentially decreased temperatures (treatment 2), the fatty acid content was significantly higher than that in the treatment 1. The aphids in treatment 2 stored relatively high level of fatty acids for a longer period of time below the threshold temperature. This result indicated that the aphids accumulate a high amount of fatty acids during the sequential decrease of temperature and provide the energy under the threshold temperature.

In summary, the accumulation of triglycerides was one of the mechanisms for cold tolerance in the pea aphids. The sequential decrease of rearing temperature make the aphids survive very well below the threshold temperature. The increased level of saturated fatty acids in the aphids reared at low temperatures is related to the reduced

turnover rather than increased level of fatty acid biosynthesis.

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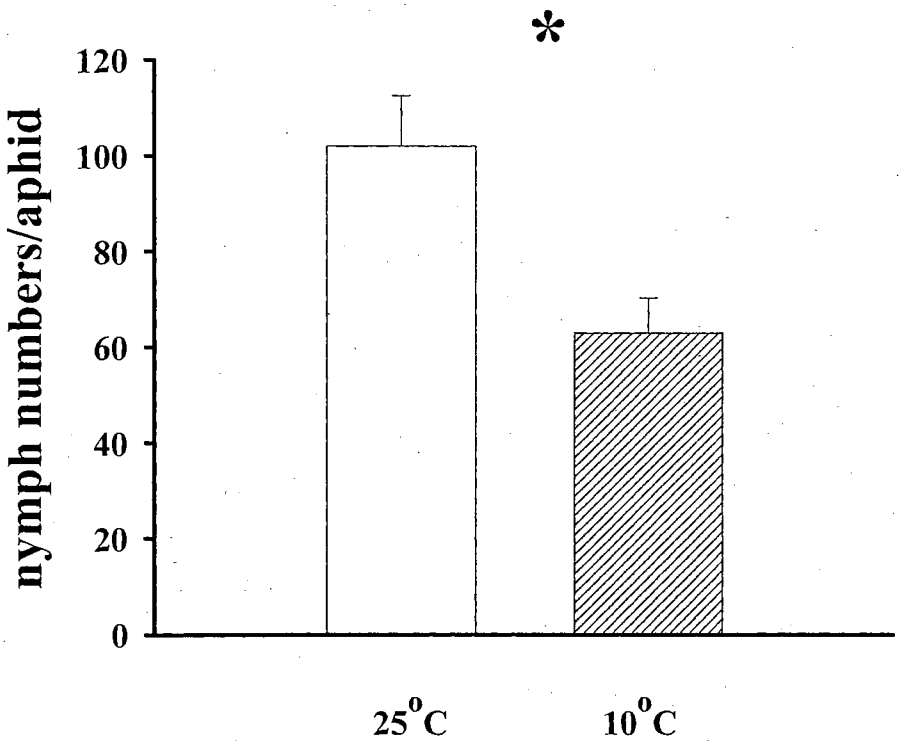

Fig. 1 Reproduction capacities of the pea aphid reared at two different tempreatures. (*) indicates significantly different, based on $p \leq 0.05$, student's *t* test. Bars represent mean$\pm$ standard deviation (n=30).

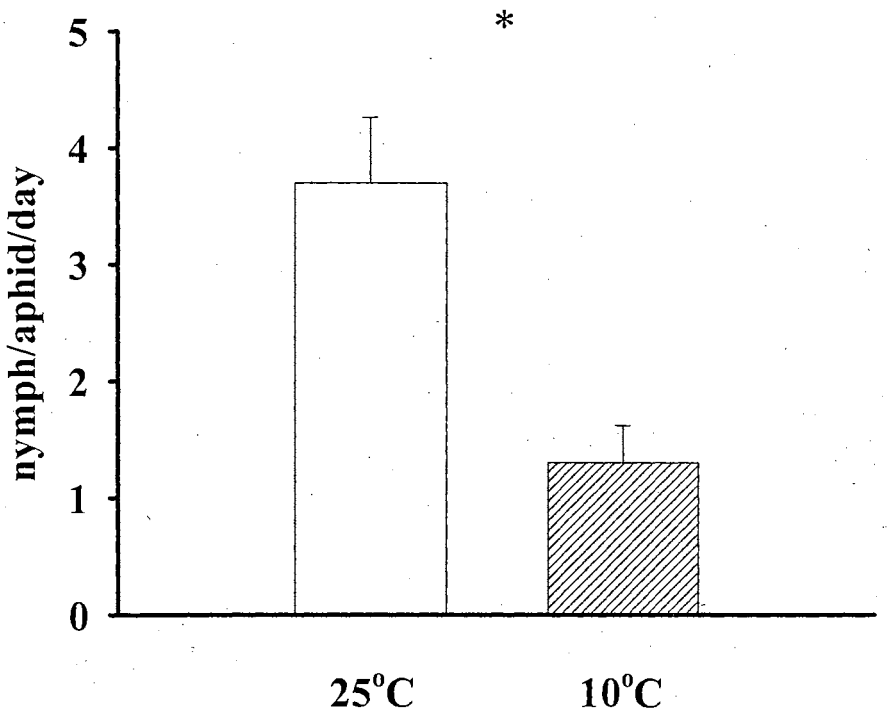

Fig. 2 Reproduction rates of the aphids reared at two different temperatures. (*) indicates significantly different,based on $p \leq 0.05$, student's *t* test. Bars represent mean±standard deviation (n=30).

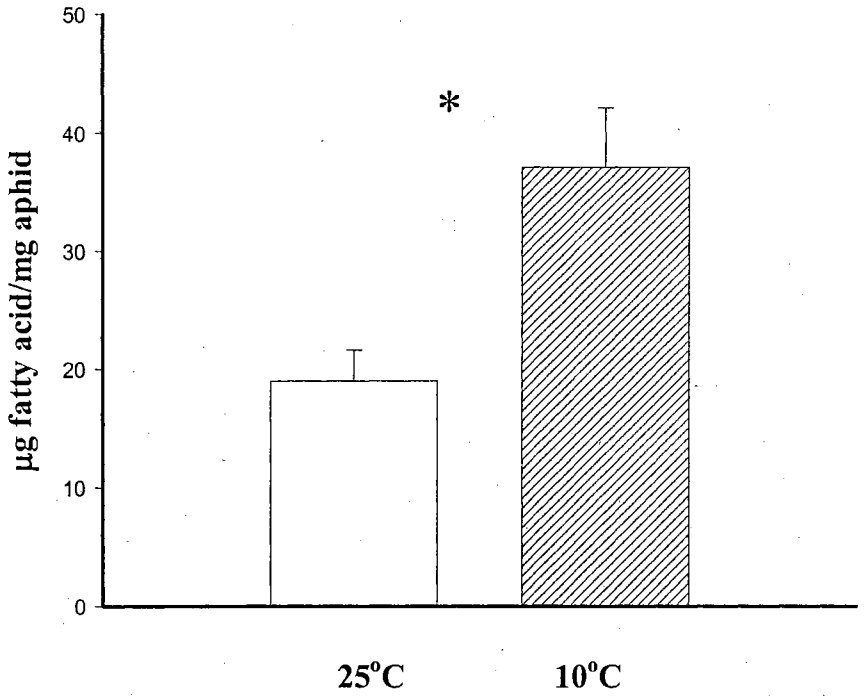

Fig. 3 Total fatty acid of pea aphids reared at 25°C and 10°C colonies. (*) indicates significant difference, based on $p \leq 0.05$, student's *t* test. Bars represent mean±standard deviation (n=3).

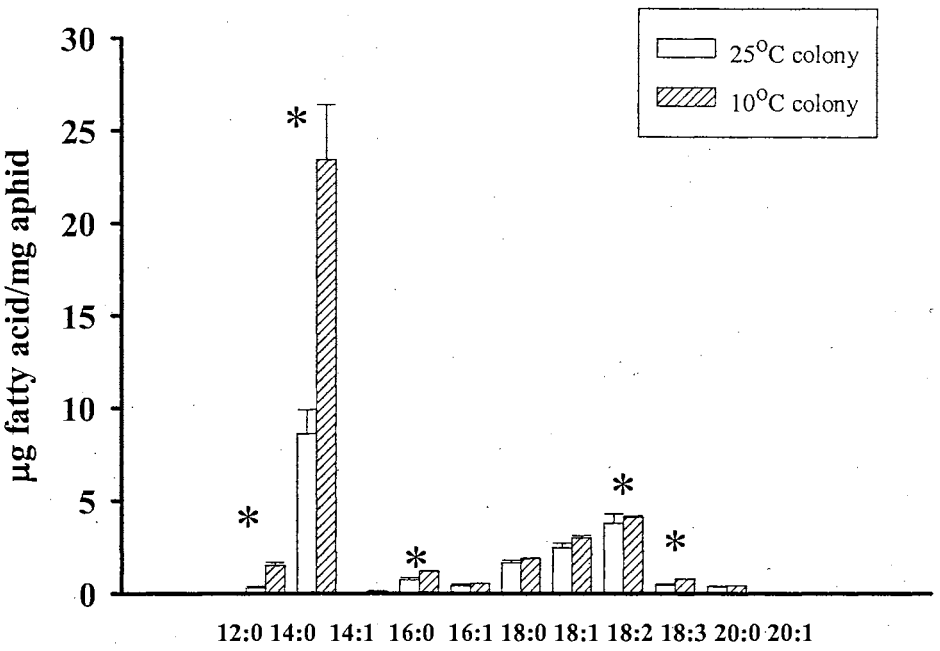

Fig. 4 Fatty acid profiles of pea aphids reared at 25°C and 10°C colonies. (*) indicates significant differences, based on $p \leq 0.05$, Student's *t* test. Bars represent mean±standard deviation (n=3).

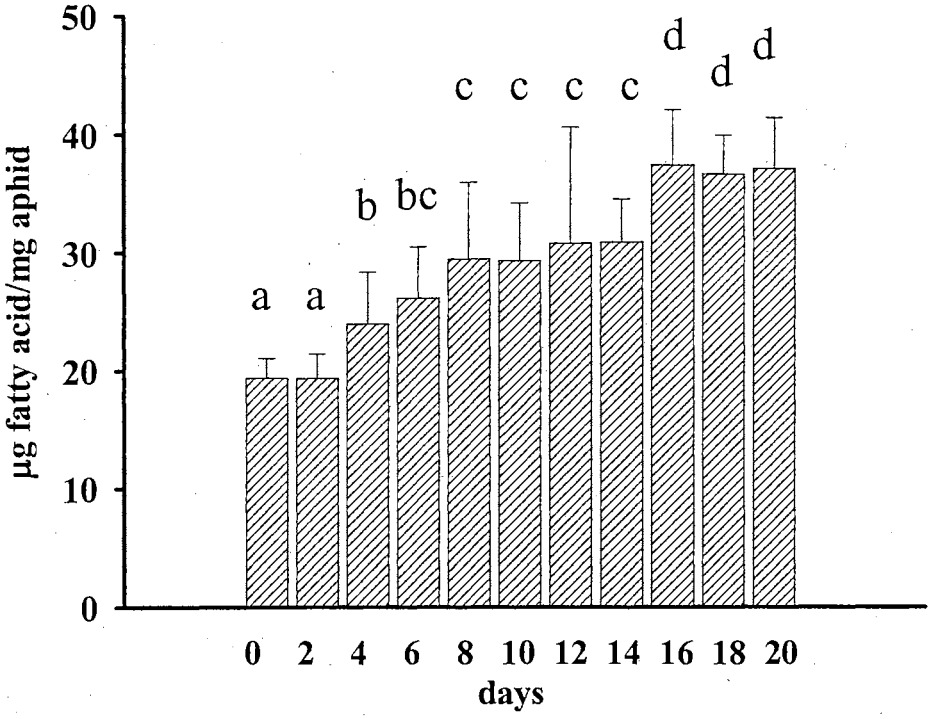

Fig. 5 Changes of total fatty acids in pea aphids under cold stress (25°C to 10°C). Different letters are indicate significantly diferent, based on $p \leq 0.05$, student's *t* test. Bars represent mean ± standard deviation (n=3).

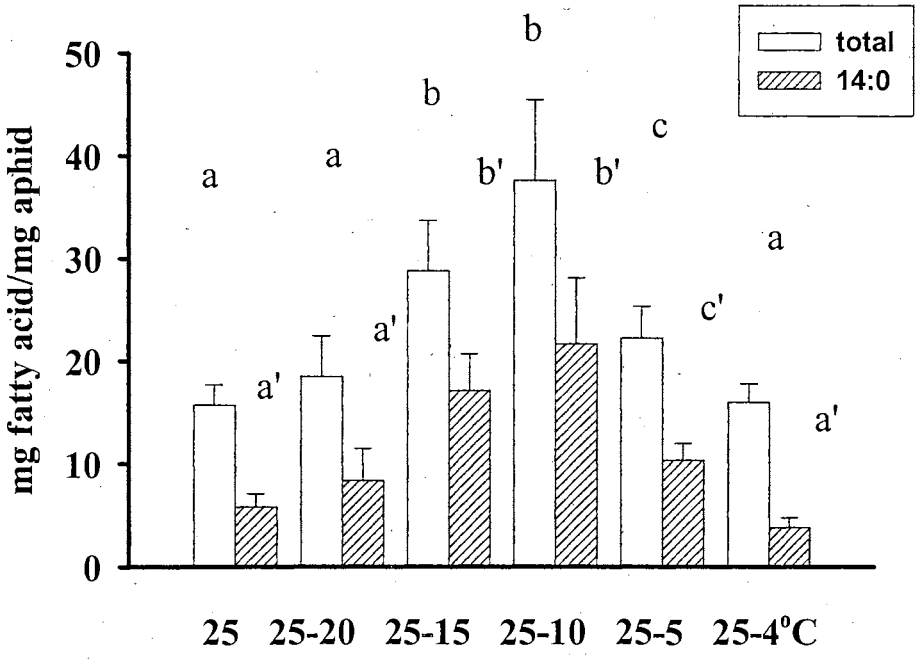

Fig. 6 Levels of total and myristic acids in pea aphids reared at different temperatures (treatment 1).The different letters indicate significant difference, based on $p \leq 0.05$, student's *t* test. Bars represent mean $\pm$ standard deviation (n=3).

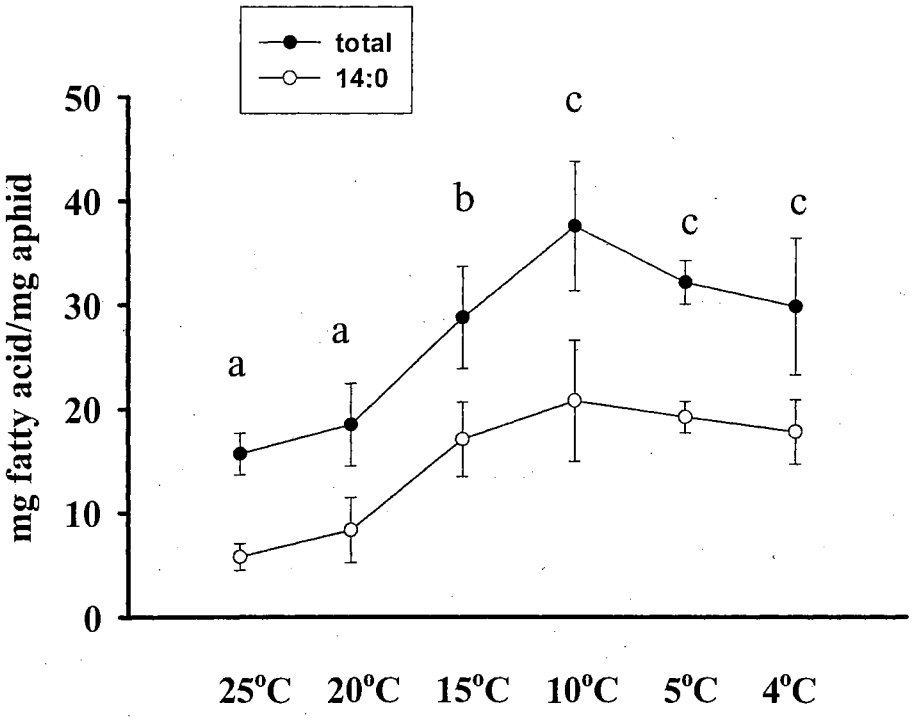

Fig. 7 Levels of total and myristic acids in pea aphids reared at different temperatures (treatment 2). The different letters indicate significantly different, based on $p \leq 0.05$, student's *t* test. Bars represent mean ± standard deviation (n=3).

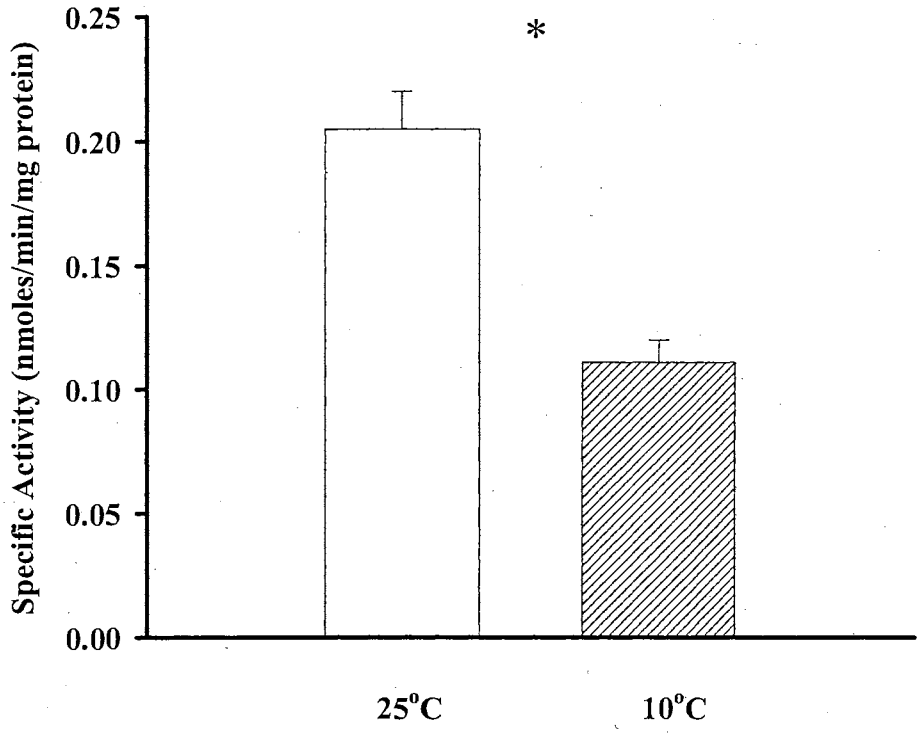

Fig. 8 Acetyl-CoA carboxylase activity in pea aphids reared at two different temperatures using acetyl-CoA as substrate. (*) indicates significantly different, student's *t* test, $p \leq 0.05$. Bars indicate mean±standard deviation (n=3).

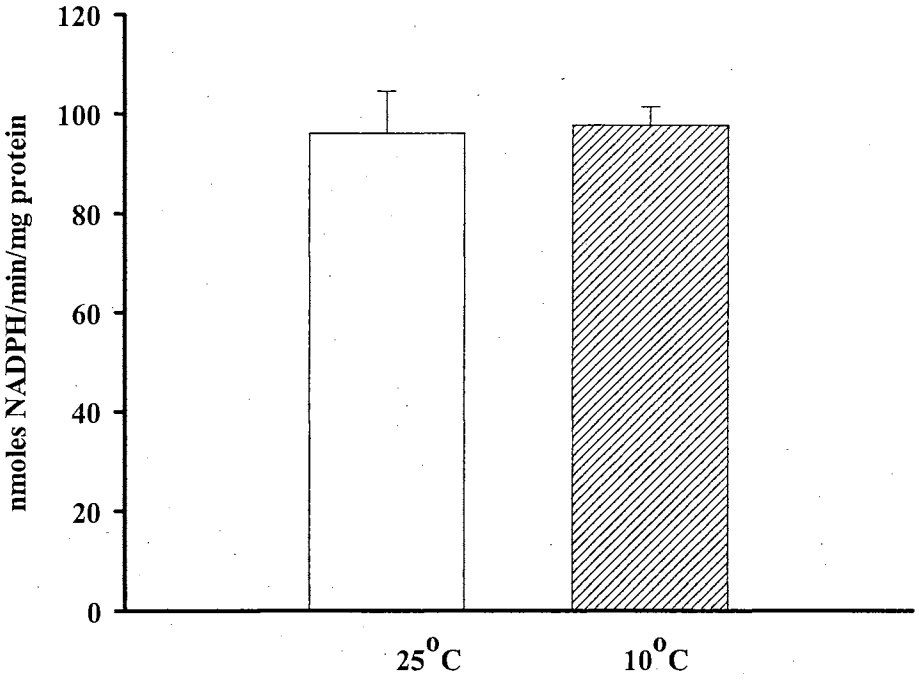

Fig. 9. Fatty acid synthase activity in pea aphids reared on two different temperatures. The FAS activities at two different temperatures were not significantly different, based on student's *t* test, $p \leq 0.05$. Bars represent mean±standard deviation (n=3).

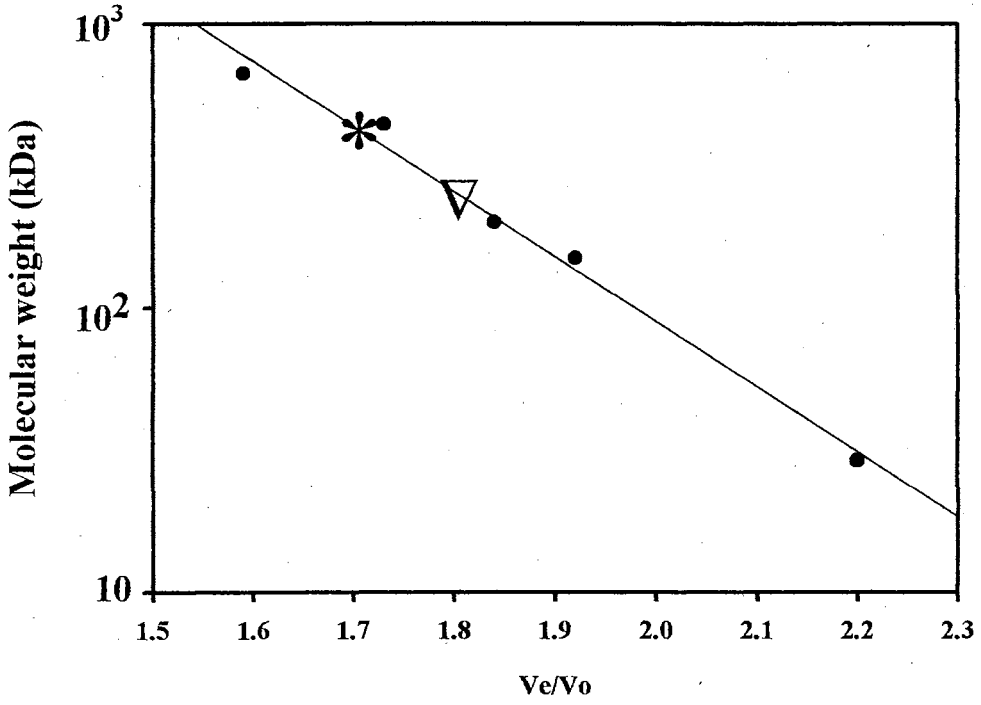

Fig. 10 Molecular weights of fatty acid synthase (FAS) determined by gel filtration in pea aphids reared at different temperatures. FAS molecular weights were 500 kDa in the aphids reared at 25°C and 10°C (*), whereas 250 kDa in aphids reared at 4°C (▽, from Brigham, 1996).

Table 1. Effect of Temperature on Fatty Acid Composition of Lipid class in the Pea Aphid[a]

	Cholesterol ester		triglyceride I		triglyceride II		triglyceride III		phospholipids	
	25°C	10°C	25°C	10°C	25°C	10°C	25°C	10°C	25°C	10°C
12:0	0	0.002±0.002	0.002±0.002	0.003±0.005	0.283±0.083	1.8±0.163*[b]	0.034±0.02	0.095±0.101	0.002±0.002	0.002±0.002
14:0	0.06±0.09	0.01±0.01	0.046±0.007	0.18±0.102*	6.45±0.911	22.75±3.04*	0.72±0.09	1.23±0.713	0.055±0.032	0.084±0.045
14:1	0.01±0.001	0	0.007±0.001	0.003±0.001	0.01±0.002	0.01±0.007	0.01±0.006	0.004±0.001	0.06±0.07	0.04±0.008
16:0	0.03±0.011	0.02±0.02	0.03±0.01	0.05±0.02	0.29±0.04	0.62±0.13*	0.06±0.004	0.03±0.004	0.14±0.047	0.18±0.033
16:1	0.08±0.06	0	0.06±0.10	0.01±0.002	0.01±0.01	0.02±0.02	0.34±0.20	0.13±0.05	0.38±0.07	0.39±0.19
18:0	0.02±0.01	0.02±0.01	0.03±0.01	0.06±0.021	0.19±0.03	0.44±0.10	0.02±0.003	0.06±0.004	1.42±0.26	1.36±0.053
18:1	0	0.001±0.003	0	0	0	0.001±0.002	0	0.001±0.00	1.90±0.28	1.98±0.13
18:2	0	0.041±0.09*	0	0.003±0.004	0.003±0.01	0.002±0.002	0	0.002±0.003	3.13±0.11	3.78±0.19*
18:3	0.01±0.01	0	0.01±0.01	0.002±0.003	0.03±0.04	0	0.01±0.01	0	0.46±0.07	0.76±0.05*
20:0	0.01±0.02	0	0.003±0.01	0.011±0.004	0	0.01±0.01	0.01±0.01	0	0.29±0.04	0.36±0.02
20:1	0	0	0	0	0	0	0	0	0.03±0.004	0.04±0.01

[a] Mean±standard deviation; unit: μg fatty acid/mg aphid (n=3).
[b] Asterisks (*) indicate significantly different between 25°C and 10°C in each column of lipid class ($p<0.05$).

Table 2. Comparison of Total Fatty Acid in the Pea Aphid under Different Treatments Over time[a]

	Treatment 1		Treatment 2	
Day	25°C→ 5°C	25°C → 4°C	10°C→ 5°C	5°C→ 4°C
Control	18.97±2.62	15.67±1.99	37.13±4.99	36.61±5.05
2	18.79±4.90	16.67±3.30	27.85±3.79	32.81±6.72
4	19.03±2.29	14.41±1.10	30.30±4.90	29.67±4.46
6	15.94±3.40	13.70±4.64	34.71±3.42	33.67±3.38
8	19.71±3.40	16.96±4.77	31.57±3.06	31.51±4.11
10	18.95±2.31	14.31±1.90	33.46±5.27	35.24±4.67
12	21.65±4.78	16.58±2.57	33.30±4.95	32.59±5.02
14	22.17±3.11	14.52±1.56	32.18±2.11	31.19±7.01
16	23.77±1.07	15.1±3.14	36.88±1.47	29.85±6.61

[a] Data represent mean±standard deviation; Unit: μg fatty acid/mg aphid (n=3).

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Effect of Precocene II on Fatty Acid Metabolism in the Pea Aphid, *Acyrthosiphon pisum* Under Cold Stress

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Abstract

Pea aphids, *Acyrthosiphon pisum,* accumulate high levels of myristic acid in triglycerides when reared at 10°C. When pea aphids were reared at 25°C, treated with precocene II at 0.5 and 2 μg per aphid, respectively, then reared at 10°C, fatty acid content increased in the aphids treated with 0.5 μg amount and did not change in the aphids treated with 2 μg precocene II compared to that in the control aphids. After the aphids were re-treated with same amount of precocene II, the fatty acid content in the aphids reared at10°C did not change in both treatments. These results showed that the precocene II could block the fatty acid accumulation in the aphids under cold stress.

Preocene II also affected reproduction of the aphids. The reproduction capacities in the aphids treated with 2 μg precocene II decreased significantly at either 25°C or 10°C compared to that in the control aphids. However, the reproduction rates of treated aphids are similar at two temperatures. It is apparent that the longevity of the aphids treated with precocene II decreased.

These results suggest that JH mediates the accumulation of myristic acid in triglycerides in pea aphids reared at low temperatures.

1. Introduction

Wiggleworth (1935) described first that juvenile hormones (JH), secreted by the corpora allata kept the insect in its larval stage in the hemipteran, *Rhodnius prolixus*. Five different juvenile hormone analogs, JH-0, JH-I, JH-II, JH-III, and 4-methyl JH-I have been identified in insects so far. Among them, JH III is the most common one. Studies in JH functions showed that JH not only keeps insects in the larva stages, but also plays an important role in regulation of development and reproduction, such as caste determination in the social insects, the regulation of behavior in honeybee colonies, the polyphenisms of aphids and locusts, regulation of larval and adult diapause, vitellogenin synthesis, and ovarian development (Nijhout, 1994; Gilbert et al. 2000).

Several studies showed that JH analogs might regulate lipid metabolism. Pfeiffer (1945) first speculated that juvenile hormone had an effect on lipid metabolism in the grasshopper. When corpora allata were removed, the lipids in the fat body were increased in many insects (Downer, 1985), whereas the amount of lipids in the fat body decreased after the corpora allata were implanted (Hill and Izatt, 1974). These result were also confirmed by the application of either JH analogs or JH agonist (JH mimic) to some insects. Topical application of JH-I in bumblebee queens induced accumulation of lipids in the fat body (Roseler and Roseler, 1988). A similar result was observed in the juvenile mud crabs, *Rhithropanopeus harrisii* (Nates and McKenneyJr, 2000). Fenoxycarb, an insect JH analog induced reduction of fatty acid content in the crab.

In other insects, removal of the corpora allata doesn't affect lipid metabolism.

Removal of corpora allata (allatectomy) didn't induce changes of lipid content in the fat body compared to control in the mosquito, *Aedes taeniorhynchus* (Wiedemann) (Van Handel and Lea, 1970) and in a cockroach, *Blaberus discodalis* (Mannix and Keeley, 1980). Neese et al. (2000) reported that JH analogs didn't affect the fatty acid content in the American dog tick, *Dermacentor variabilis* (Say) and in *Ornithodoros parkeri* Cooley.

Contrary to the above descriptions, application of JH agonists (methoprene) and JH antagonists (precocene II) induced different results. Application of methoprene in the larvae of the eastern spruce budworm, *Choristoneura fumiferana* (Clemens) induced accumulation of lipids in fat body (Mulye and Gordon, 1993). Application of methoprene (JH mimic) caused accumulation of fatty acid content in the pea aphid (Neese, 1995).

Although studies have been done in effects of JH analogs on fatty acid metabolism in several species of insects, little is known about the effect of precocene II on fatty acid metabolism as it relates to rearing temperature in aphids. The objective of the present study was to characterize the effect of precocene II on fatty acid metabolism at different rearing temperatures in the pea aphids. Understanding how JH antagonists affect fatty acid metabolism in pea aphids in response to rearing temperature will provide important information about pea aphid physiology and may provide clues for better control of aphids pests.

2. Materials and Methods

2.1. Aphid colony

Pea aphids, *Acyrthosiphon pisum* (Harris) were reared on faba bean (Vicia faba L.) cv. 'Windsor' maintained at 25°C with a photoperiod of 16:8.

2.2. Application of precocene II to the pea aphids

Apterous healthy aphids were treated with JH antagonist, precocene II dissolved acetone: DMSO: ethanol in a ratio of 90:5:5 (v/v/v). Precocene II (2.5 μg/μl and 10 μg/μl) was applied topically to pea aphids (0.2 μl/aphid). Untreated and solvent treated aphids were used as controls. After the treatments, the aphids were reared at 10°C for 16 days in the 1st experiment. In the 2nd experiment, the treated aphids were reared at 25°C and 10°C for 24 h then retreated with same amount of precocene II. Then the aphids were moved to 10°C for 16 days. Aphid samples consisted of 10 aphids of uniform size and at least three replicates were collected. The samples were weighed immediately and stored at -20°C until use for fatty acid analysis.

2.3. Lipid extraction

Aphids were homogenized by hand with glass/glass homogenizers in 3.8mls of a methanol: chloroform: water (2:1:0.8) solution and the lipids were extracted according to Bligh and Dyer (1959). 50μl heptadecanoic fatty acid methyl ester (17:0, 1μg/μl) were added to each sample as an internal standard. Lipid extracts were stored at -20°C in chloroform containing 0.5% (w/v) butylated hydroxytoluene (BHT).

2.4. Fatty acid analysis

Lipid from total extracts were saponified with methanolic-potassium hydroxide, followed by transmethylation with methanolic-boron trifluoride (Shipley et al., 1993). Fatty acid methyl esters were analyzed by gas chromatography (GC) on a HP5890 Series II gas chromatograph with HP7673 autosampler (Hewlett Packard, Santa Clarita, CA). FAMEs were introduced onto a DB-225 column 30mx0.25mm with a 0.15μm film thickness (J&W Scientific, Folsom, CA) using a split injector set at 250°C with a 1:10 split ratio.

Ultrapure helium was the carrier gas at 1ml/min and the GC program was as follows: 120°C for 2 min, 10°C/min to 200°C, 5°C/min to 225°C. A flame ionization detector, operating at 300°C, was used and peak areas were recorded by HP-Chemstation software. FAME peaks were identified by comparison of retention times with authentic FAMEs (NuCheck Prep, Inc., Elysian, MN).

2.5. Reproduction rate

Virgin apterous adult aphids were obtained by rearing nymphs of the same size (same age) from 25°C colony. Aphids were treated with precocene II (2 μg/aphid) described as above. Untreated aphids were used as the controls. Then the aphids were reared at 25°C and 10°C in 3 replicates with 10 aphids each. The nymphs were counted every 2 days from the aphids reared at 25°C and every 5 days from the aphids reared at 10°C.

2.6. Analysis of data

Statistical analysis of data was carried out using the Student's *t* Test.

3. Results

3.1. Total fatty acid content of aphids in response to precocene II treeatment

Total fatty acid in pea aphid reared at 25°C did not change after being treated with 2 μg precocene II/aphid as compared to untreated aphids. It apparently decreased after treatment with 0.5 μg of precocene II, but the difference was not statistically different ($p>0.05$) (Fig. 1). When precocene II treated aphids were reared at 10°C, the total fatty acids in untreated, solvent treated, and 0.5 μg treated aphids increased about 2-fold compared to 25°C control insects, whereas pea aphids treated with 2 μg precocene II

didn't change as compare to insects at 25°C (Fig. 1). The changes in total fatty acids of pea aphids under different treatments reflected the changes of myristic acid, which exclusively occurred in triglyceride fraction (Fig. 2). Myristic acid in untreated, solvent treated, and 0.5 μg treated aphid reared at 10°C increased 2-fold compared to 25 °C control and it did not change when the aphids were treated with 2 μg precocene II under same condition. When the aphids were retreated with same amount of precocene II and reared at 10°C, the fatty acid accumulation was blocked even at 0.5 μg/aphid (Fig. 3). The unsaturated fatty acids in each of two precocene II treatments decreased compared to controls (Fig. 4).

3.2. Reproduction of aphids in response to precocene II treatment

Aphids treated with precocene II produced fewer nymphs as compared to untreated controls either at 25°C or 10°C (Fig. 5). The reproduction rates, however, were quite similar between treated and control aphid (Fig. 6). It was apparent that the aphid longivity was affected by precocene II treatment.

4. Discussion

We report here the effect of precocene II on fatty acid content in the pea aphid, *Acyrthosiphon pisum* in relation to rearing temperature. The mechanisms for fatty acid changes in relation to removal or implantation of corpora allata in insects is not clearly understood yet. Beenakkers (1983) speculated that accumulation of fatty acids in response to removal of the corpora allata was related to the prevention of egg ripening, increased or constantly high feeding activity, and increased lipid synthesis in fat body. Chinzei et al. (1981) described that vitellogenin contained about 8% lipid in the migratory locust, *Locusta migratoria* L. and these lipids could be stored in the fat body

after prevention of egg ripening. This may not be a plausible mechanism. According to Hagedorn and Kunkel (1979), yolk synthesis is not directly controlled by JH in all insects. On the other hand, accumulation of lipids in the fat body was also observed in male insects after removal of the corpora allata (Odhiambo, 1966; Walker and Bailey, 1971a). So, accumulation of lipids in fat body is not wholly correlated with the prevention of egg ripening.

The accumulation of lipid in the fat body is closely related to the increased level of lipogenesis (Neese, 1995). More [1- ^{14}C] acetate was incorporated in allatectomized female cockroaches as compared to control insects (Vroman et al. 1965). In the desert locust, Walker and Bailey (1971b) noted a similar result. They found higher [1- ^{14}C] acetate incorporation into the fat body of allatectomized locusts. All these processes should be related to a specific enzyme activity in lipogenesis. In the allatectomized male cockroaches, glucose-6-phosphate dehydrogenase activity increased (Walker and Bailey, 1971c). Keeley (1978) also described a similar result in an allatectomized migratory locust. He noted that specific activities of lipogenic enzymes such as ATP-citrate lyase and glucose-6-phosphate dehydrogenase increased two to three times in allatectomized insects.

Fatty acid content of the pea aphids treated with precocene II at certain concentrations did not change when they were reared at 10°C compared to control. This result showed that precocene II did affect accumulation of fatty acid in the treated aphids under cold stress. It is not likely that increased level of lipogenesis occurred in this circumstance. However, the exact mechanism of precocene II treatment in pea aphids is still unknown. These data suggest that aphids may be unique in their regulation of lipid

metabolism with respect to JH. How precocene II blocks fatty acid biosynthesis in the aphids will be investigated in our future research.

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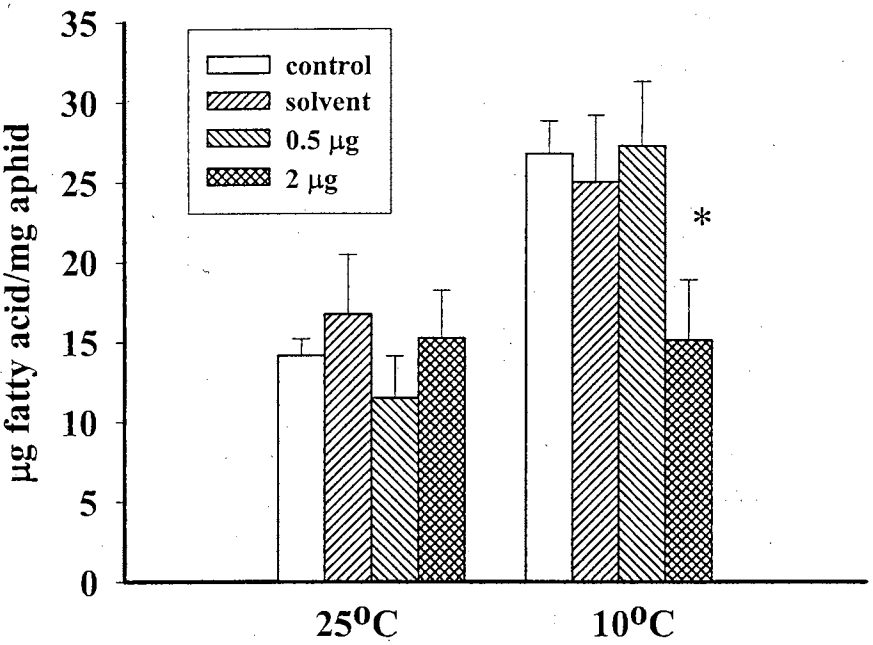

Fig. 1 Total fatty acid content of pea aphids in response to precocene II treatment. Aphids were treated once with indicated materials and were then reared at either 25°C or 10°C for 16 days prio to fatty acid analysis. (*) indicates significant differences, based on $p \leq 0.05$, student's *t* test. Bars represent mean ± standard deviation (n=3).

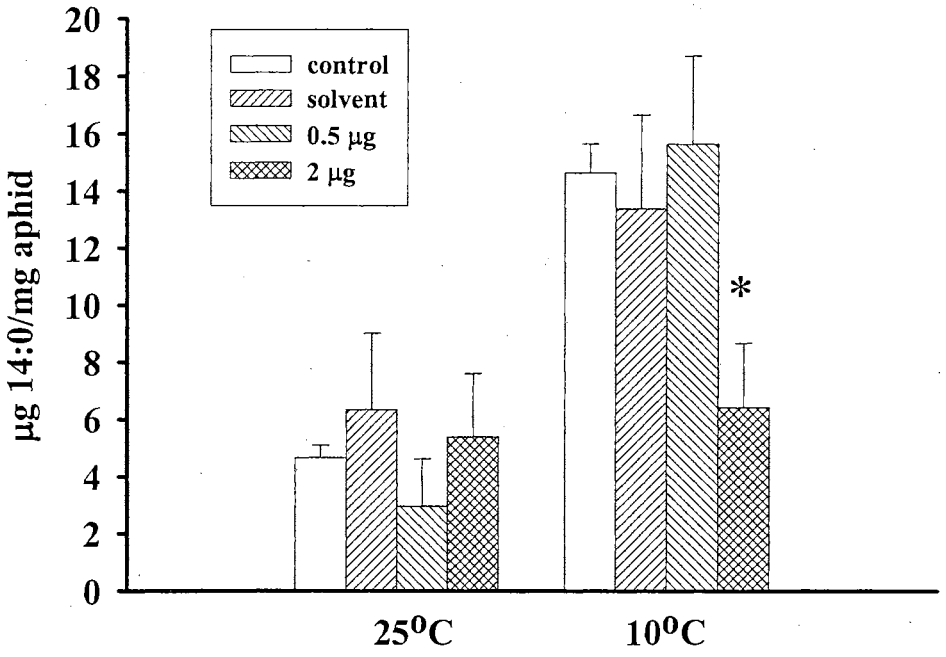

Fig. 2 Myristic acid content of pea aphids in response to precocene II treatment. Aphids were treated once with indicated materials and were then reared at either 25°C or 10°C for 16 days prio to fatty acid analysis. (*) indicates significant differences, based on $p \leq 0.05$, student's *t* test. Bars represent mean ± standard deviation (n=3).

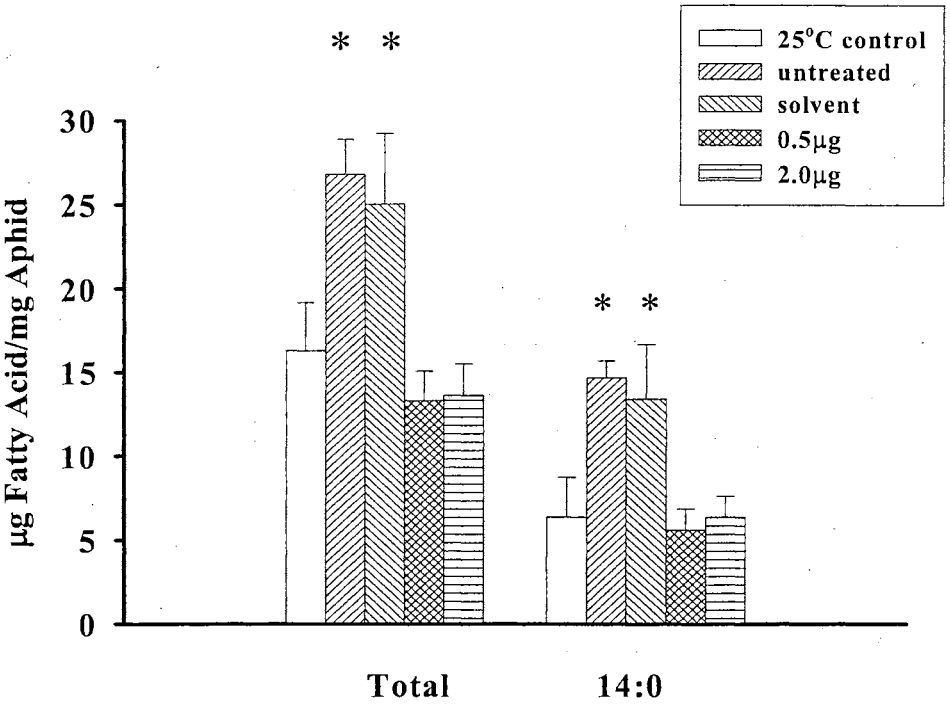

Fig. 3 Total fatty acid and myristic acid contents of pea aphids in response to precocene II retreatment (Experiment 2). Aphids were treated twice with indicated materials and were then reared at 10°C for 16 days prio to fatty acid analysis. (*) indicates significant differences, based on $p \leq 0.05$, student's *t* test. Bars represent mean ± standard deviation (n=3).

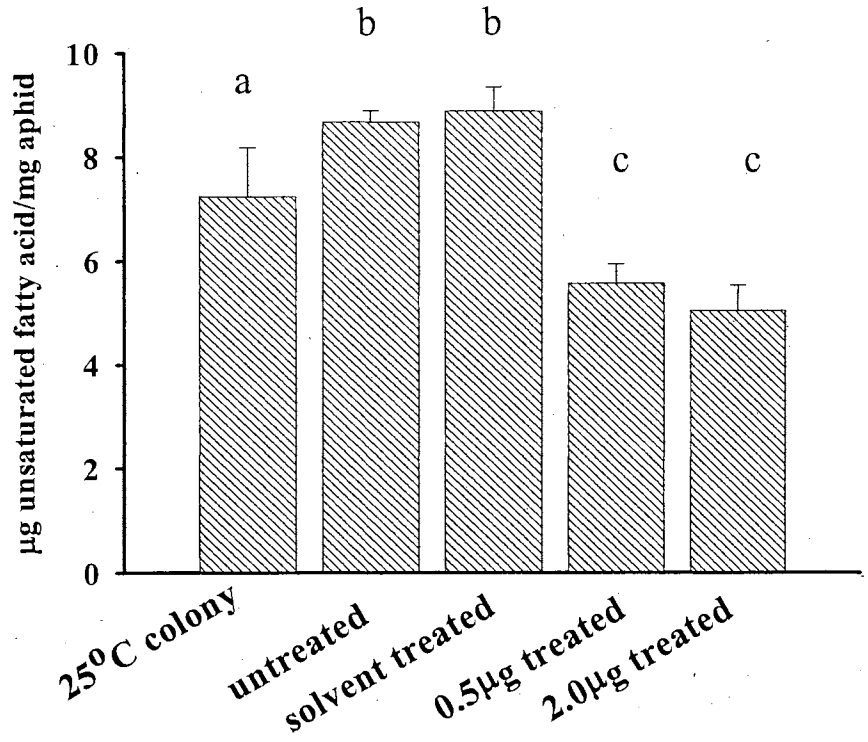

Fig. 4 Changes of total unsaturated fatty acids in pea aphids reared at 10°C in response to precocene II retreatments. Different letters indicate significant differences, based on $p \leq 0.05$, student's *t* test. Bars represent mean±standard deviation (n=3).

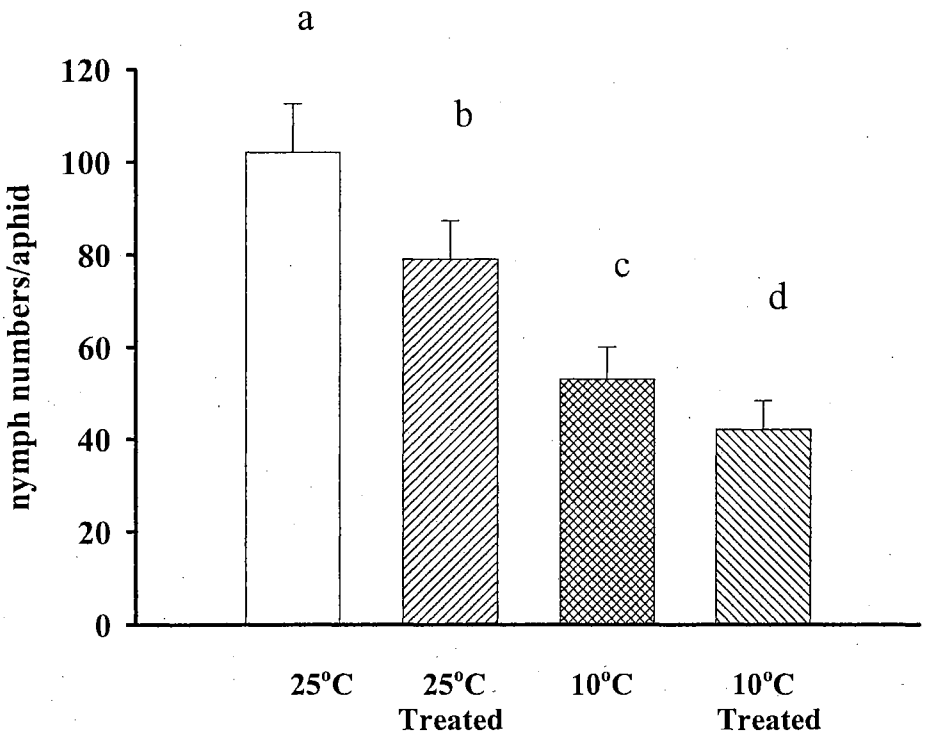

Fig. 5 Reproduction capacities of pea aphids in response to precocene II (2 μg/aphid) treatment and temperatures. The different letters indicate significantly different, based on $p \leq 0.05$, student's *t* test. Bars represent mean ± standard deviation (n=30).

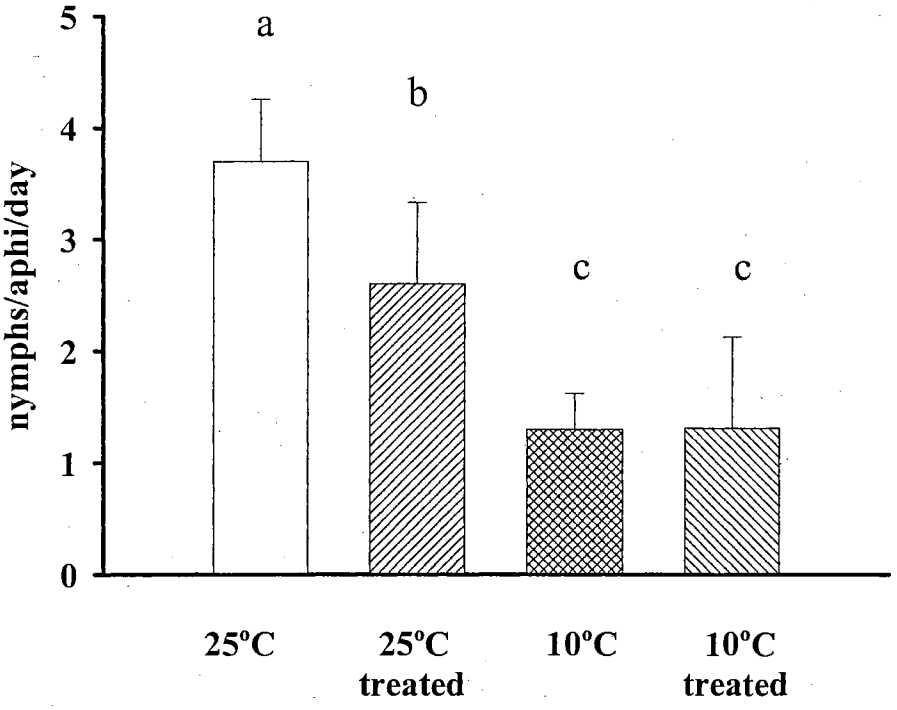

Fig. 6 The reproduction rate of the pea aphid in response to precocene II treatment (2 μg/aphid). The different letters indicate significantly different, based on $p \leq 0.05$, student's *t* test. Bars represent mean ± standard deviation (n=30).

VI. Prepared for Insect Biochemistry and Molecular Biology

Cold Induced Changes in Unsaturated Fatty Acid Content and Δ9-Desaturase Expression in the Pea Aphid, *Acyrthosiphon pisum*

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Abstract

Fatty acid analysis by gas chromatography in the pea aphid, *Acyrthosiphon pisum* showed that the aphids had increased levels of unsaturated fatty acid when reared under cold stress. Two Δ9-desaturase cDNA fragments were amplified by primers based on the known Δ9-desaturase genes from a pea aphid salivary gland cDNA library. These fragments were sequenced and were 52.2% identical at the amino acid level. One fragment designated Ap1 showed 62-78% amino acid identity to known Δ9-desaturases. The second designated Ap2 showed 51-59% identity. Two specific primers were designed based on the pea aphid Δ9-desaturase cDNA fragment sequences. RT-PCR using these primers showed activation of Δ9-desaturase at the transcriptional level in aphids reared at 10C. However, Δ9-desaturase enzymatic activity in the aphids at low temperature did not increase. The slight increase in unsaturated fatty acids in aphids reared at 10°C may be related to lower reproduction rates at lower temperatures.

1. Introduction

Any organism has the potential ability to modify fatty acid composition of its membrane phospholipids in response to changes in environmental temperature (Marcartney et al., 1994) to maintain the proper membrane fluidity during the cold stress (Sinensky, 1974; Hochachka and Somero, 1984; Hazel, 1985; Nakashima et al., 1996). Membrane fluidity depends mainly on the ratio of saturated/unsaturated fatty acyl chains of the phospholipids. Cold stress in some insects is known to induce changes in fatty acid composition, particularly an increase in unsaturated fatty acids, which mainly occur in phospholipids (Keith, 1966; Baldus and Mutchmor, 1988; Ohtsu et al., 1993; Ohtsu et al., 1998). Low temperature acclimation causes a decrease in the amounts of 3 saturated fatty acids (myristic, pentadecanoic, and palmitic) and an increase in the amounts of 3 unsaturated fatty acids (linoleic, eicosadienoic, and arachidonic) in the nerve cord extract of American cockroaches (Baldus and Mutchmor, 1988). Palmitoleic acid increased dramatically in diapausing face flies compared to nondiapausing flies (Valder et al., 1969). Phospholipids of many cold-tolerant fruit flies contained more unsaturated fatty acid than cold susceptible organisms. The percentage of unsaturated fatty acids increased with a decrease in rearing temperature in fruit flies (Ohtsu et al., 1993 & 1998). These changes probably contribute to maintaining the homeoviscosity of the cellular membranes, thereby increasing cold tolerance.

Δ9-desaturase, which places a double bond between C9 and C10 position of long chain saturated fatty acids plays an important role in the process. This oxidative reaction

is catalyzed by the iron-containing, microsomal enzyme, and involves cytochrome b_5, NADH or NADPH-cytochrome b_5 reductase, and molecular oxygen. In the reaction, the preferred substrates are palmitoyl- and stearoyl-CoA, which are converted into palmitoleoyl- and oleoyl-CoA, respectively. Δ9-desaturase generally contains 300-500 amino acid residues and has two potential transmembrane regions and three conserved histidine cluster motifs (one HXXXXH and two HXXHH) (Shanklin et al., 1994).

Among the acyl-CoA desaturases, genes encoding Δ9-desaturase homologs have been cloned and sequenced in different organisms, including fungi, bacteria, yeast, protozoan, arthropods, and vertebrates. In arthropods, the genes of tick (Luo et al., 1997), house cricket (Riddervold et al.*), fruit fly (Wicker-Thomas et al., 1997), the cabbage looper moth (Liu et al., 1999), redbanded leafroller (Liu et al.*), European corn borer (Liu et al.*), corn earworm (Rosenfield and Knipple*), and silkworm (Yoshiga et al., 2000) have been cloned and sequenced and finally the amino acid sequences deduced.

Studies have shown that the mechanisms of the cold adaptation in organisms are related to both Δ9-desaturase gene expression and its activity. In the process, gene expression was increased in response to cold temperature and was due in part to transcriptional activation in a carp (Tiku, et al., 1996) and in a protozoan, *Tetrahymena thermophila* (Nakashima et al., 1996). In *T. thermophila,* transcription of the desaturase gene was increased 2.0-fold at 23°C and 2.6-fold at 15°C compared to that of 35°C (Nakashima et al., 1996). The amount of Δ9-desaturase mRNA was low in warm acclimated carp and increased greatly after 48 hours cooling. After 3 to 5 days it reached the highest level (Tiku et al., 1996). Then it decreased gradually over time, but maintained a relatively high level. Cold induced Δ9-desaturase expression in carp was

also related to posttranslational effects (Tiku et al., 1996). They noted that a preexisting latent desaturase became activated 24 to 48 hours after initiation of cooling. The release of sequestered enzyme or activation by posttranslational modification was involved in the process. This effect might be an early response to moderate cooling, whereas transcriptional up-regulation and increased desaturase protein might require much lower temperature. The elevation of the desaturase mRNA was also related to its stability (Nakashima et al., 1996; Tiku et al., 1996). Nakashima et al. described that the half-life of the desaturase mRNA in cells growing at 35°C was less than 45min, whereas at 15°C it was more than 60min.

The other mechanism of increased level of unsaturated fatty acid is related to Δ9-desaturase activity. Its activity was also affected by various factors. In male houseflies, desaturase activity increased about 26-fold from emergence until the insects were three and four days old. In females, the activity increased about 12-fold from emergence until day 2, then it sharply decreased on day three and maintained 6-8-fold increase for the next four to five days. After 11 days, the activity had decreased to the value observed at emergence in both sexes (Wang, et al., 1982). A similar result was reported by Cripps et al. (1988) in the house cricket. When the carp was progressively cooled from 30 to 23, 17, and 10°C, the saturated/unsaturated fatty acid ratio decreased linearly (Tiku et al., 1996). They observed that Δ9-desaturase activity increased 2.5-fold at 23°C on day one and there were no changes in the amount of desaturase protein or transcript. At 17°C, desaturase activity was greatly increased and reached 7-fold on day five and desaturase transcription increased 10 to 20-fold. At 10°C, transcription of desaturase gene increased 40 to 50-fold and desaturase activity did not increase compared to that of 17°C. (Tiku, et

al., 1996; Trueman, et al., 2000). When soybean seeds were grown at 20°C, stearol-acyl carrier protein desaturase activity was increased compared to that at 35°C (Cheesbrough, 1990).

Although Δ9-desaturase genes have been cloned and sequenced in several insect species so far, little is known about the mechanisms of cold adaptation in insects. The objective of the present study was to characterize the biochemical and molecular mechanisms of unsaturated fatty acid metabolism in pea aphid in response to different rearing temperatures.

2. Materials and Methods

2.1. Insects

Pea aphids, *Acyrthosiphon pisum* (Harris) were reared on faba bean (Vicia faba L.) cv. 'Windsor' maintained at 25°C with a photoperiod of 16:8. A second colony was maintained at 10°C using the same plants and photoperiod. In the 1st treatment, pea aphids from the 25°C colony were reared on faba bean at 20°C, 15°C, 10°C, 5°C and 4°C respectively. In the 2nd treatment, the aphids were reared at the same temperatures as first one, however, the temperature was decreased gradually and the aphids were infested from the aphids reared at higher temperature (such as 25→ 20→ 15→10→ 5→ 4°C). Aphids were transferred to the lower temperature after 16 days. Aphids were sampled every 2 days (10 aphids/sample, 3 samples). In the time course study, the aphids were reared at 10°C from 25°C colony and sampled at 6 h, 12 h, 1 day, 2 days, 4 days, 8 days, and 16 days (10 aphids/sample, 3 samples). The healthy apterous aphids were sampled in each experiment.The samples were weighed and then stored frozen at –20°C until fatty acid analysis.

2.2. Analysis of unsaturated fatty acid

2.2.1. Lipid extraction

Aphids were homogenized by hand with glass/glass homogenizers in 3.8 ml of methanol: chloroform: water(2:1:0.8, v/v) and the lipids were extracted according to Bligh and Dyer (1959). 50 µl heptadecanoic fatty acid methyl ester (17:0, 1 µg/µl of chloroform) was added to each sample as an internal standard. Lipid extracts were stored in chloroform containing 0.5% (w/v) butylated hydroxytoluene (BHT) at –20°C .

2.2.2. Fatty acid analysis

Lipid from total extracts was saponified with methanolic-potassium hydroxide, followed by transmethylation with methanolic-boron trifluoride (Shipley et al., 1993). Fatty acid methyl esters were analyzed by gas chromatography (GC) on a HP5890 Series II gas chromatograph with HP7673 autosampler (Hewlett Packard, Santa Clarita, CA). FAMEs were introduced onto a DB-225 column 30 m × 0.25 mm with a 0.15 µm film thickness (J&W Scientific, Folsom, CA) using a split injector set at 250°C with a 1:10 split ratio. Ultrapure helium was the carrier gas at 1ml/min and the GC program was as follows: 120°C for 2 min, 10°C/min to 200°C, 5°C/min to 225°C. A flame ionization detector, operating at 300°C, was used and peak areas were recorded by HP-Chemstation software. FAME peaks were identified by comparison of retention times with authentic FAMEs (NuCheck Prep, Inc., Elysian,MN).

2.3. Fatty acid desaturase activity assay

2.3.1. Isolation of microsomes

Isolation of microsomes from the pea aphids reared at different temperatures was based on the methods of Wang et al. (1982) and Batcabe et al (2000).

2.3.2. Acyl CoA desaturase activity

The stearoyl-CoA desaturase activity of aphid microsomes was determined by a modification of the method described by Wang et al. (1982) and Batcabe et al. (2000). The reaction was carried out in 1 ml of 100 mM Tris-HCl, containing 20 µlStearoyl-CoA [50 µM, including 0.00363 µmol/ml radiolabeled (Specific activity: 55mCi/mmmol) and 0.0464 µmol/ml unlabeled stearoyl-CoA] and 0.5 mM NADPH (20 µl). The reaction was started by adding 1 mg of microsomal proteins and the mixture incubated at 37°C for 8 min. as described by Wang et al. (1982) and Batcabe et al. (2000). The reactions were stopped by adding 0.5 ml 5% KOH in methanol and then heated at 65°C for 90 min or at 100°C for 20 min. The mixtures were acidified with HCl and fatty acid extraction and methylation were as described by Bligh and Dyer (1959). The distribution of radioactivity between the saturated and unsaturated fatty acids was determined by silver ion chromatography (Christie, 1989). The ability of the silver ion columns to separate fatty acids according to degree of saturation was confirmed by separating fatty acid standards followed by analysis by gas chromatography.

2.4. Molecular cloning

2.4.1. Design of primers

The amino acid sequences of the putative Δ9 desaturase of arthropods (tick and insect) and other organisms (vertebrates, protozoan, fungi, bacteria, and plants) were compared by sequence alignment. Two pairs of oligoprimers were designed based on conserved domains.

Δ9F1: 5'TGCACCSAYAARTAYTCNGARAC3
Δ9R1: 5'GCAGTYTTRTARTCCCANGGRAA3'
Δ9F2: 5'CACAAYGCNCGNGGYTT3'

Δ9R2: 5'GGGAANGTRTGRTGRTARTTRTG3'

2.4.2. Isolation of cDNA from pea aphid salivary gland cDNA library (λTRIPCEX2).

Pea aphid salivary gland cDNA library was provided by Dr. Jerry Reeck and Dr. John Reese (Kansas State University). λDNA was isolated from the cDNA library using Wizard^{R} Plus Miniprep DNA Purification System kit (Promega).

2.4.3. Polymerase chain reaction (PCR)

The following mixture was prepared in a 0.5 ml microcentrifuge tube on ice. 1 μl (20 ng) of DNA template, 5 μl (10 pmole/μl) forward primer, 5 μl (10 pmole/μl) reverse primer, 5 μl 10 × amplification buffer, 5 μl (2.5 mM) dNTP, 2.5 μl (2.5 mM) $MgCl_2$, 0.5 μl Taq DNA polymerase, and added ddH_2O to a final volume of 50 μl. In the first PCR, the library DNA was used as the template and Δ9F1/R1 as primers. In the second PCR reaction, the first reaction product was used as the template and Δ9F2/R2 as primers. The mixtures were overlaid with 50 μl mineral oil and amplified in a thermal cycler (MJ Research, USA). The Taq DNA polymerase was added last after the temperature reached 94°C. The mixture was kept at 94°C for 3 min, then 40 cycles of at 94°C for 30 sec, at 45°C for 45 sec, at 72°C for 45 sec and extension at 72°C for 10 min.

2.4.4. Recovery and purification of DNA

The amplified DNA was separated by electrophoresis on 1% agarose gel (Sambrook et al., 1989). The PCR product at the expected size was recovered using NucleoSpin Nucleic Acid Purification Kits (Clontech).

2.4.5. Subcloning of the DNA fragment

The purified DNA was ligated to the pGEM^{R}–T Vector using pGEM-T and pGEM-T Easy Vector Systems (Promega). Then the ligated DNA was transformed into DH5α^{TM}

competent cells following the instructions of the manufacturer (Invitrogen Life Technologies). Plasmids with insert were isolated using the Qiagen spin column mini-prep kit and sequenced at the Recombinant DNA/Protein Core Resource Facility at Oklahoma State University, using the automated DNA sequencer. GenBank search was performed using BlastX (Altschul et al. 1990).

2.5. Reverse transcriptase PCR and northern blot analysis

2.5.1. Total RNA extraction and mRNA isolation

Total RNA was prepared from the pea aphid, *Acyrthosiphon pisum*, from two different colonies using the RNAgents^{R} Total RNA Isolation System (Promega). mRNA was isolated from the total RNA using MicroPoly(A) Purist^{TM} mRNA purification kit (Ambion).

2.5.2. RT-PCR

A pair of specific primers, Δ9F3/R3 and Δ9F4/R4 were designed in terms of the sequenced fragments. In each of two PCR reaction tubes, 25 μl Reaction Mix, 1 μl template (total RNA of 25°C or 10°C colony), 1 μl forward primer, 1 μl reverse primer, 1 μl each actin* forward (5'-CGTGATATCAAGGAGAAATTGTGC-3') and reverse (5'-CAGAGAAGCCAAGATGGATCCTC-3') primers (which could amplify 424 bp actin gene fragment; Dr. Jerry Reeck and Dr. John Reese,Kansas State University, provided pea aphid actin cDNA sequence.) were mixed and then 19 μl ddH_2O was added. PCR was performed under the following conditions: 20 min at 50°C, 2 min at 94°C, 40 cycles of 15 sec at 94°C, 30 sec at 55°C, 72°C for 1 min; and 10 min at 72°C, overnight at 4°C. 5 μl of product was sampled at 20, 25, 30, 35, and 40 cycles, respectively.

2.5.3. Northern blot analysis

Ten micrograms of total RNA each from 25 and 10°C colonies were run on a 1% agarose gel in 6.6 M formaldehyde/1 × MOPS buffer. RNA was transferred onto a Zeta-Probe^{R} Blotting nitrocellulose membrane (Bio-Rad), and the membrane was incubated in prehybridization solution (5 × SSC/ 5× Denhardt's/ 0.1% SDS) at 55°C for 1h. The sequenced DNA fragments (457 and 404 bp) and actin were used as probes. The probes were labeled with [α-^{33}P] dCTP by using Multiprime DNA Labeling System (Amersham RPN 1601), and the heat denatured probe was added to the prehybridization solution and incubated at 55°C overnight. After the incubation, the membrane was washed in 0.1 × SSC/ 0.1% SDS twice for 10 min, twice for 20 min, twice for 30 min, and twice for 1h at 58°C, and exposed to Pierce CL-X Posure^{TM} film. The density of each band was measured on a GS-710 densitometer (Bio-Rad).

2.6. Analysis of data

The statistical analysis of data was carried out using the Student's *t* Test.

3. Results

3.1. Unsaturared fatty acid analysis

The total unsaturated fatty acid of 10°C colony aphids was significantly higher than that of aphids reared at 25°C (Fig. 1). 18:3 fatty acid in the aphids reared at 10°C increased 2-fold compared to that of aphids at 25°C. In both treatments, unsaturated fatty acids increased significantly over time at low temperatures (Fig. 2 & 3). A significantly higher amount of unsaturated fatty accumulated in the aphids reared at 5°C and 4°C in treatment 2 compared to treatment 1 (Fig. 2 & 3).

3.2. Fatty acid desaturase activity assay

Δ9-desaturase activity in the pea aphids reared at 10°C was significantly lower than that in the aphids reared at 25°C (Fig. 4). Increased level of unsaturated fatty acid in the aphids reared at 10°C does not appear to be due to an increased level of Δ9-desaturase activity. When the mixtures were incubated at 10°C for 8 min, the specific activities of Δ9-desaturase in the aphids reared at 25°C and 10°C were same as the above result. This suggests that the incubation temperature does not affect the Δ9-desaturase specific activity.

3.3. Molecular cloning and northern blot analysis

3.3.1. Isolation and sequencing of Δ9-desaturase cDNA fragment

Two degenerate primers, Δ9F1/R1 and Δ9F2/R2 were used for the cloning of the pea aphid Δ9-desaturase gene fragments. λDNA template and Δ9F1/R1 primers were used in the first PCR and the first reaction product was used as a template and Δ9F2/R2 as primers in the second reaction (Fig. 5). The gene fragments were subcloned into pGEM^{R}-T Vector. The clones from the first reaction were labeled from 1-1 to 1-8, whereas clones from the second reaction from 2-1 to 2-9 (Fig. 6). Among them, 1-1,1-3, 1-5, 1-7, 2-2, 2-5, 2-6, and 2-9 were digested with two restriction enzymes, Pst I and Sph I (Fig. 7). Then 1-7, 2-5, and 2-6 were selected and sent for sequence analysis. Expected sizes of cDNA fragments were obtained from 1-7, 2-5, and 2-6 corresponding to 457, 404, and 404bp, respectively. Through the sequence alignments, we found that 2-6 sequence matched perfectly to 1-7, however, 2-5 sequence did not. 2-5 shared 60.9% identity with the 2-6 sequence. The deduced amino acid sequences were obtained based on the nucleotide sequences. The identity of amino acid sequences between 1-7 (as Ap1 in Table1) and 2-5 (as Ap2 in Table1) was 52.2%. The amino acid sequence identities among various

desaturases are shown in Table1. Ap1 showed 62-78% identity to other desaturases, whereas Ap2 showed about 51-59% identity to others. At this point, we do not know why the identity between Ap1 and Ap2 is so low. Based on the sequence alignment, we designed two pairs of specific primers:

Δ9F3 (5'-TAGCTGATCCGATTGTTTCCT-3')
Δ9R3 (5'-CAAAGCTAATAAAGCTACAGT-3')
Δ9F4 (5'-GAAATGGATCCCTACGTCATG-3')
Δ9R4 (5'-GCCCCAGCCCGAACATGGACACG-3')

corresponding to 1-7 (2-6) and 2-5, respectively. The cross-reactions are shown in Fig. 8. The PCR products were obtained from Δ9F3/R3 primers with 1-7 template and Δ9F4/R4 primers with 2-5 template, respectively. The results showed the specificity of the two pairs of primers.

3.3.2. Extraction of total RNA and RT-PCR

1.26 mg and 2.53 mg total RNA was extracted from 100 aphids reared at 25 and 10°C respectively. Total RNA of the aphids reared at 10°C was increased about 2-fold compared to that of the aphids at 25°C (9.58 μg/mg aphid vs 5.95 μg/mg aphid). The concentration of RNA from 10°C aphids was equalized with that of 25°C aphids and 1μl template was used in each reaction. Actin was used as a control in the reactions. Δ9F4/R4 were used as primers. Δ9F3/R3 did not amplify a product in the RT-PCR under any condition including different annealing temperatures and different substrate concentrations. The PCR bands from 10°C sample at 30, 35, and 40 cycles were much stronger than those of 25°C colony, whereas the actin bands from the 2 colonies were similar (Fig. 9). This result indicated that Δ9-desaturase gene expression increased in response to low rearing temperature. We tried to confirm the result by Northern Blot. However, we did not find the Δ9-desaturase mRNA by using ^{33}P labeled probes. Actin

mRNA was detected from either total RNA or mRNA. This result indicates that the amount of Δ9-desaturase mRNA is possibly too low to detect in the Northern Blot.

4. Discussion

In the present study, we report the changes of unsaturated fatty acid content in the pea aphid, *Acyrthosiphon pisum*, and some biochemical and molecular adaptation under cold stress. Unsaturated fatty acid in the pea aphids reared at low temperature increased significantly. These changes may play an important role in maintaining suitable membrane fluidity at low temperature (Sinensky, 1974). Our result is consistent with the studies in a protozoan, *Tetrahemena thermophila* (Nakashima, et al., 1996) and a carp (Tiku, et al., 1996). In the carp, this adaptation is due in part to an increased activity of fatty acid desaturases. Δ9-desaturase introduces the first double bond into saturated fatty acids, such as palmitic or stearic acid. The acyl-CoA desaturase activity increased significantly under cold stress in the carp (Tiku, et al., 1996) and the protozoan (Nakashima, et al., 1996).

It is surprising that Δ9-desaturase specific activity in the pea aphids reared at 10°C decreased about 2-fold compared to that in the pea aphids reared at 25°C. The increased level of unsaturated fatty acids in the aphids under cold stress is possibly related to some other enzymes such as Δ12-desaturase. Aphids are one of the insects that have been shown to be able to carry out Δ12 desaturation of fatty acids (de Renobales et al., 1990). Linoleic acid (18:2) increased significantly in the phospholipid fraction whereas oleic acid (18:1) did not. We are currently in the process of determining the activity of Δ12-desaturase. The 18:3 in the pea aphid has an unusual structure ($\Delta^{9, 12, 17}$-

18:3) (Dillwith et al., 1993). Pea aphids are unable to synthesize this fatty acid from acetate and it is not clear if it is synthesized from other precursors obtained from the host plant. Host plants do not contain this 18:3 isomer (Dillwith et al., 1993).

The studies in the carp and the protozoan showed that increased levels of unsaturated fatty acid under cold stress were due in part to desaturase activation at the transcriptional level. Based on the sequence alignment among known Δ^9-desaturases, we designed two pairs of oligoprimers, which were used in the amplification of pea aphid salivary cDNA library. It is surprising that we cloned and sequenced two different desaturase cDNA fragments. The identity between the deduced amino acid sequences of the two fragment is 52.2%. It is relatively low compared to identity of the Δ9-desaturase isomers in same species. For example, the identity between two Δ9-desaturase isomers in *Bombyx mori* was up to 98% (Yoshiga et al., 2000). Comparisons of Ap1 with other desaturase corresponding portions showed that the identities are much higher than Ap2 versus other desaturases (Table 1). Although the two deduced amino acid fragments hit the Δ^9-desaturase isomers, the exact functions of the two isomers must be clarified in future studies. In terms of the sequence alignment between the two fragments, we designed two specific primers for reverse-transcriptase PCR (RT-PCR). A pair of specific primers (Δ^9 F3/R3) corresponding to AP1 did not produce a product under any RT-PCR whereas Δ9 F4/R4 corresponding to Ap2 did. The RT-PCR result showed that the amount of Δ9 -desaturase mRNA in the aphids reared at 10°C is much higher than that in the aphids reared at 25°C. This result is consistent with studies in other organisms, but is not consistent with the activity assay for Δ9-desaturase. One possible explanation is that the Ap2 clone is a Δ12-desaturase gene fragment rather than a Δ9-desaturase. Δ12-desaturase

has never been cloned from an animal. Therefore at this time we cannot draw comparisons to the fragment cloned. We estimate we have the sequence for approximately 40% of the Ap2 desaturase and future studies will be aimed at obtaining the complete sequence and expressing the protein for functional assay.

Acknowledgment

This project was supported by a Graduate assistantship from the Department of Entomology and Plant Pathology,

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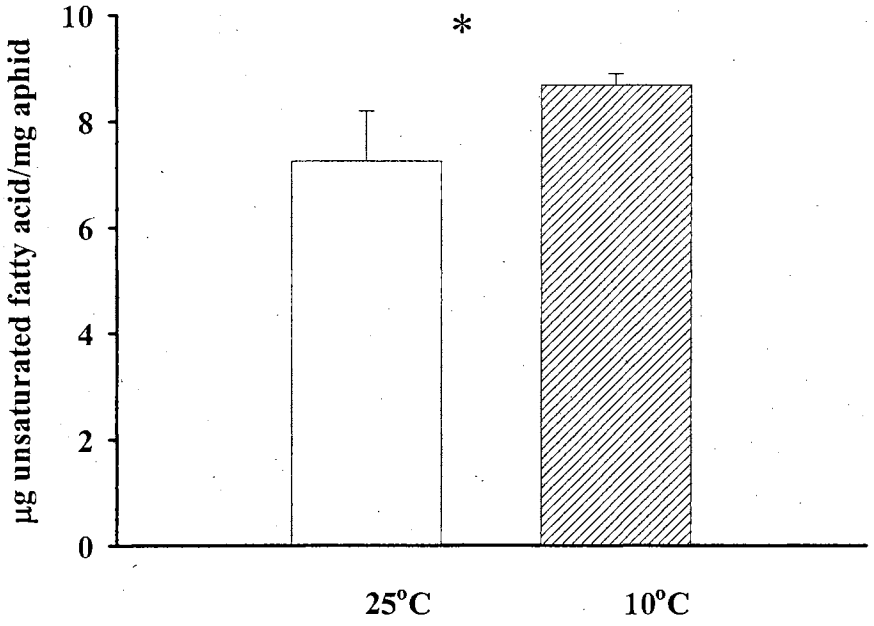

Fig. 1 Total unsaturated fatty acids of pea aphids reared at 25°C and 10°C colonies. (*) indicates significantly different, based on $p \leq 0.05$, student's *t* test. Bars represent mean±standard deviation (n=3).

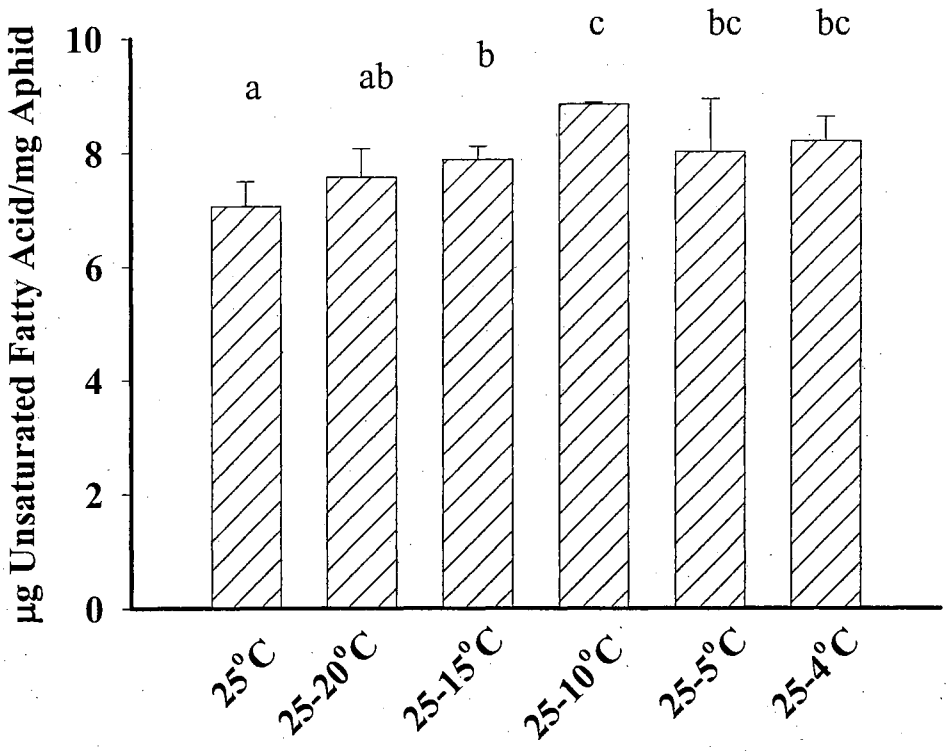

Fig. 2 Levels of total unsaturated fatty acid in pea aphids reared at different temperatures (Treatment 1). The different letters indicate significant differences, based on $p \leq 0.05$, student's *t* test. Bars represent mean ± standard deviation (n=3).

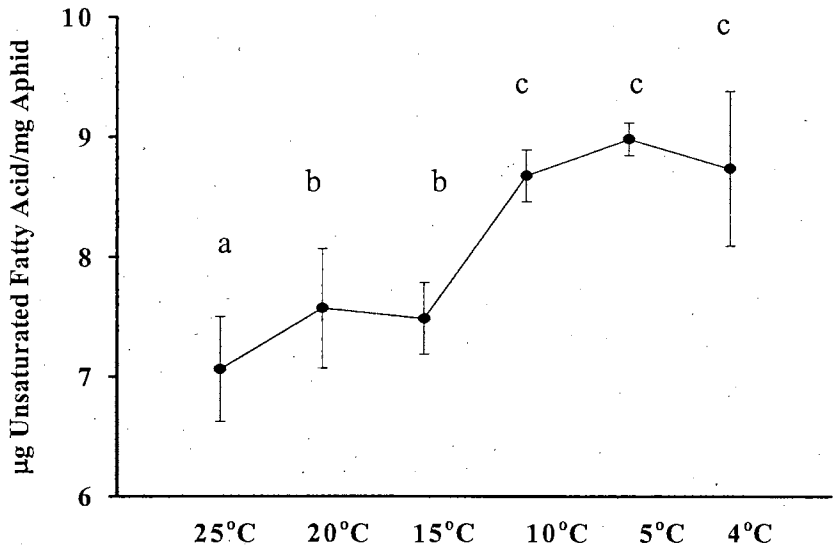

Fig. 3 Levels of total unsaturated fatty acid in pea aphids reared at different temperatures (Treatment 2). The different letters indicate significant differences, based on $p \leq 0.05$, student's *t* test. Bars represent mean ± standard deviation (n=3).

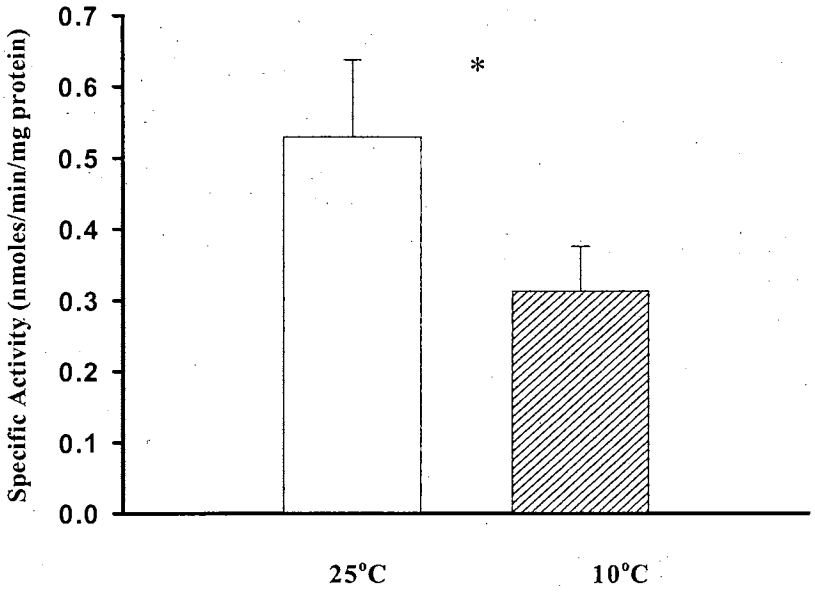

Fig. 4 Δ9-desaturase activity in pea aphids reared at two different temperatures. (*) indicates significantly different, based on $p \leq 0.05$, student's t test.Bars represent mean $\pm$ standard deviation (n=3).

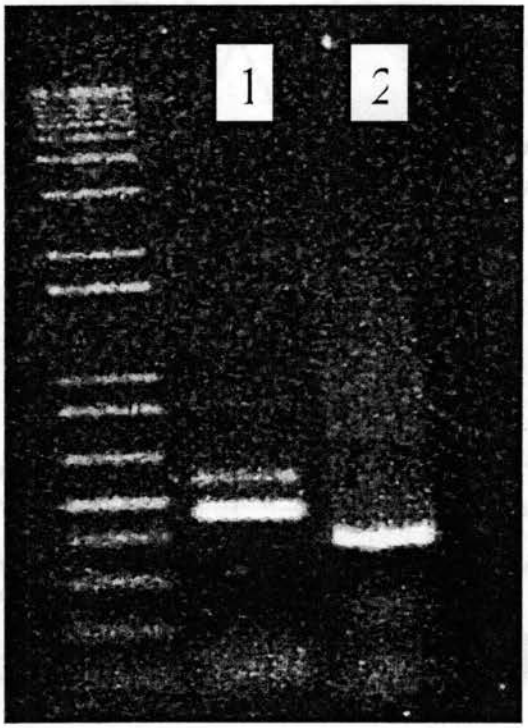

Fig. 5 The amplification of cDNA fragment of Δ9-desaturase from pea aphids by PCR. Lane 1: λcDNA used as a template and Δ9F1/R1 as primers. Lane 2: first reaction product as a template and Δ9F2/R2 as primers. Bands 1 and 2 correspond to 454 and 404bp, respectively.

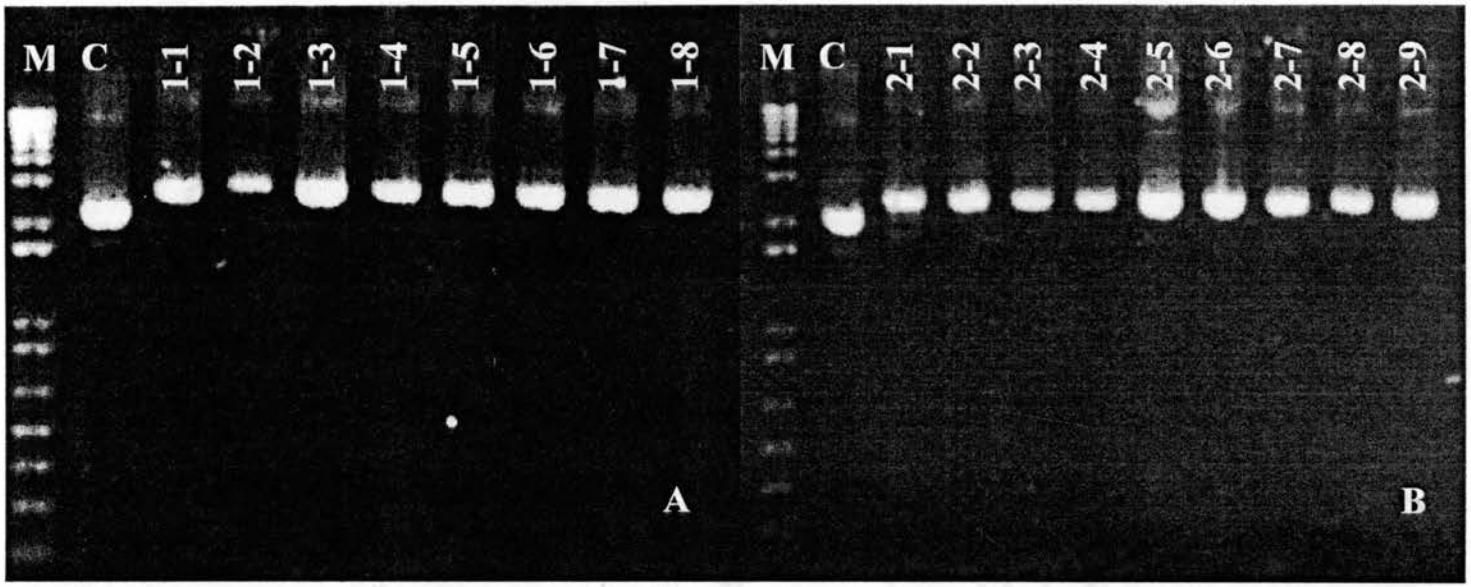

Fig. 6 Verification of transformation. A: 1-1 to1-8, plasmids with insertions, which were amplified by 9F1/R1 primers; B: 2-1 to 2-9, plasmids with insertions, which were amplified by 9F2/R2. M: DNA marker; C: control.

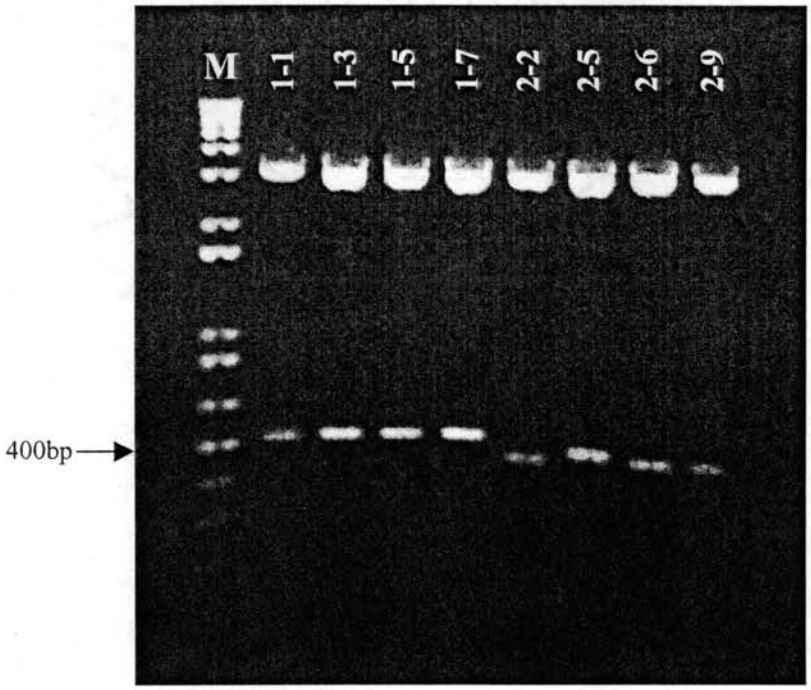

Fig. 7 Digestion of subcloned cDNA fragments. The plasmids were selected from A and B of Fig. 6 and digested by two restriction enzymes, Pst I and Sph I; M: DNA marker.

Fig. 8 Primer specificity. Two pairs of primers, Δ9F3/R3 and Δ9F4/R4 were designed in terms of sequence alignment. In lane 1 and 2, 1-7 was used as templates and in lane 3 and 4, 2-5 as templates; In lane 1 and 3, Δ9F3/R3 as primers and in lane 2 and 4, Δ9F4/R4 as primers.

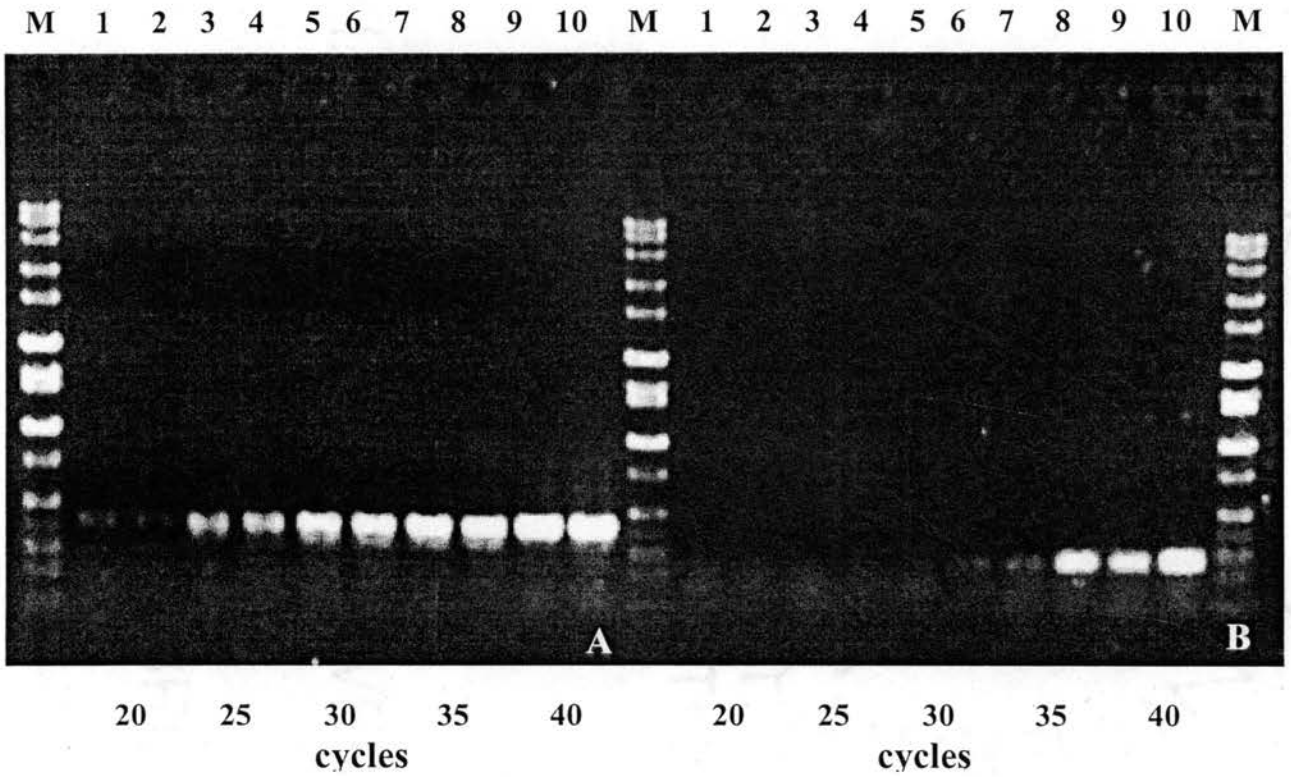

Fig 9. RT-PCR for Δ9-desaturase gene expression (B) at two different temperatures. Actin (A) was used as a control. Odd number samples were from 25°C colony and even number samples from 10°C colony. 9F4/R4 were used as primers. The PCR products were sampled at 20,25, 30, 35, and 40 cycles, respectively. M: DNA marker.

Amino acid identities among various desaturases (%)

	Ap2	Ad1	Av1	Hz2	On	Bm1	Tn1	Dm1	Aa	Mm	Hs
Ap1	52.2	74.2	72.9	74.2	73.5	66.9	68.9	77.5	62.3	64.9	64.2
Ap2		56.7	56.0	58.2	59.0	55.2	55.2	52.2	52.2	52.2	51.5
Ad1			66.0	66.0	65.0	55.0	60.0	62.0	51.0	49.0	51.0
Av1				85.0	86.0	63.0	65.0	64.0	52.0	49.0	50.0
Hz2					86.0	63.0	63.0	67.0	52.0	50.0	48.0
On						63.0	64.0	66.0	51.0	50.0	50.0
Bm1							79.0	56.0	49.0	50.0	47.0
Tn1								59.0	50.0	50.0	50.0
Dm1									44.0	47.0	47.0
Aa										51.0	50.0
Mm											84.0

Ap: *Acyrthosiphon pisum*: Ad: *Acheta domestica*; Av: *Argyrotaenia velutinana*; Hz: *Helicoverpa zea*; On: *Ostrinia nubilalis*; Bm: *Bombyx mori*; Tn: *Trichoplusia ni*; Dm: *Drosophila melanogaster*; Aa: *Amblyomma americanum*; Mm: *Mus musculus*; Hs: *Homo sapien.*

VII. Summary and Conclusions

Aphids, such as greenbug, *Schizaphis graminum*, survive very well during winter in Oklahoma. How these aphids survive under cold temperature is an interesting question. The pea aphid, *Acyrthosiphon pisum*, was used as a model species in my study to understand the biochemical and molecular mechanisms involved in the fatty acid metabolism under cold stress.

Total fatty acid including saturated fatty acid and unsaturated fatty acid in the pea aphid reared at low temperatures increased significantly compared to that at high rearing temperature. This change is mostly reflected in the large increase of myristic acid, which exclusively occurs in triglycerides.

The studies on enzyme activity including acetyl –CoA carboxylase (ACCase) and fatty acid synthase (FAS) showed that the increased level of saturated fatty acid in the aphids reared at low temperatures is not due to increased levels of fatty acid biosynthesis. Our result with ACCase assay indicated that the aphids at 25°C have a capacity to biosynthesize more fatty acid than the aphids in 10°C. Fatty acid synthase activities in the aphids from 25°C and 10°C are quite similar.

The result in the investigation of reproduction showed that the reproduction rate of the aphids at 10°C decreased dramatically compared to aphids at 25°C. Studies by others indicate that the developmental rate of the aphids at 10°C decreased 5-fold compared to aphids in 25°C (Lamb, 1992). It is likely that the aphids produce triglycerides for both reproduction and energy storage. Therefore, the increased level

of saturated fatty in the aphids reared at low temperature is probably the result of a decrease in the amount of lipid used for reproduction and not increased biosynthesis.

Treatment of pea aphids with precocene II, a juvenile hormone antagonist affects the fatty acid composition compared to the control. This result strongly suggests that JH does regulate the fatty acid metabolism in the pea aphids. The regulation of fatty acid metabolism in aphids is probably unique compared to the findings in other insect species. In most insects, removal of corpora allata caused increased level of fatty acids in fat body (Downer, 1985).

Our studies on unsaturated fatty acids in the pea aphids show that the aphids produce more unsaturated fatty acid from saturated fatty acid under cold stress. This appears to be an increase in transcription of a desaturase (presumably Δ9-desaturase). However this increase in transcription does not result in a corresponding increase in Δ9-desaturase activity. It is possible that the probe produced to measure Δ9-desaturase activity is really for a Δ12-desaturase. Future studies will be needed to confirm this possibility.

Related to the project, further studies should be conducted. First, evaluation of tritrophic interactions based on fatty acid level in the aphid. Secondly, biochemical and molecular mechanisms in the fatty acid metabolism in the aphids treated with precocene II. Lastly, based on the two cDNA fragments, full sequences of desaturases should be obtained. Furthermore, functional assays should also be conducted.

VIII. Appendix

Figures, which are related to and support the results in my manuscripts, are included in the section. There are 22 figures including color, weight, ovariole number, fatty acid profiles of lipid separation in the aphids, fatty acid content in the aphids under different treatments, Δ9-desaturase sequence alignment, and fatty acid separation by siver ion chromatography.

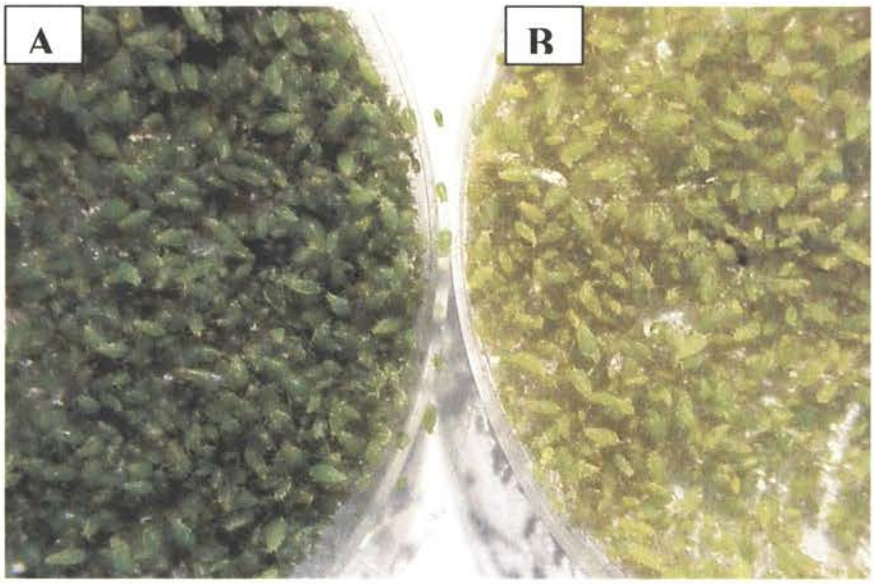

Fig.1 The color of the aphids at 25°C (B) and 10°C (A) colonies.

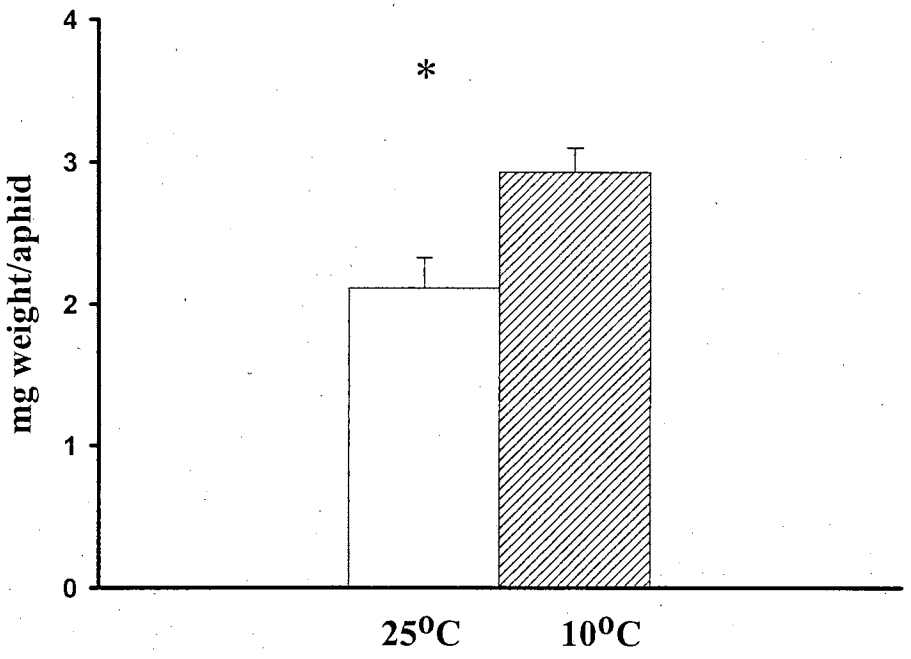

Fig. 2 Weights of pea aphids reared at 25°C and 10°C colonies. (*) indicates significant difference, based on $p \leq 0.05$, student's *t* test. Bars represent mean±standard deviation (n=75).

Fig. 3 Ovarioles of a pea aphid from 25°C colony magnified 30×.

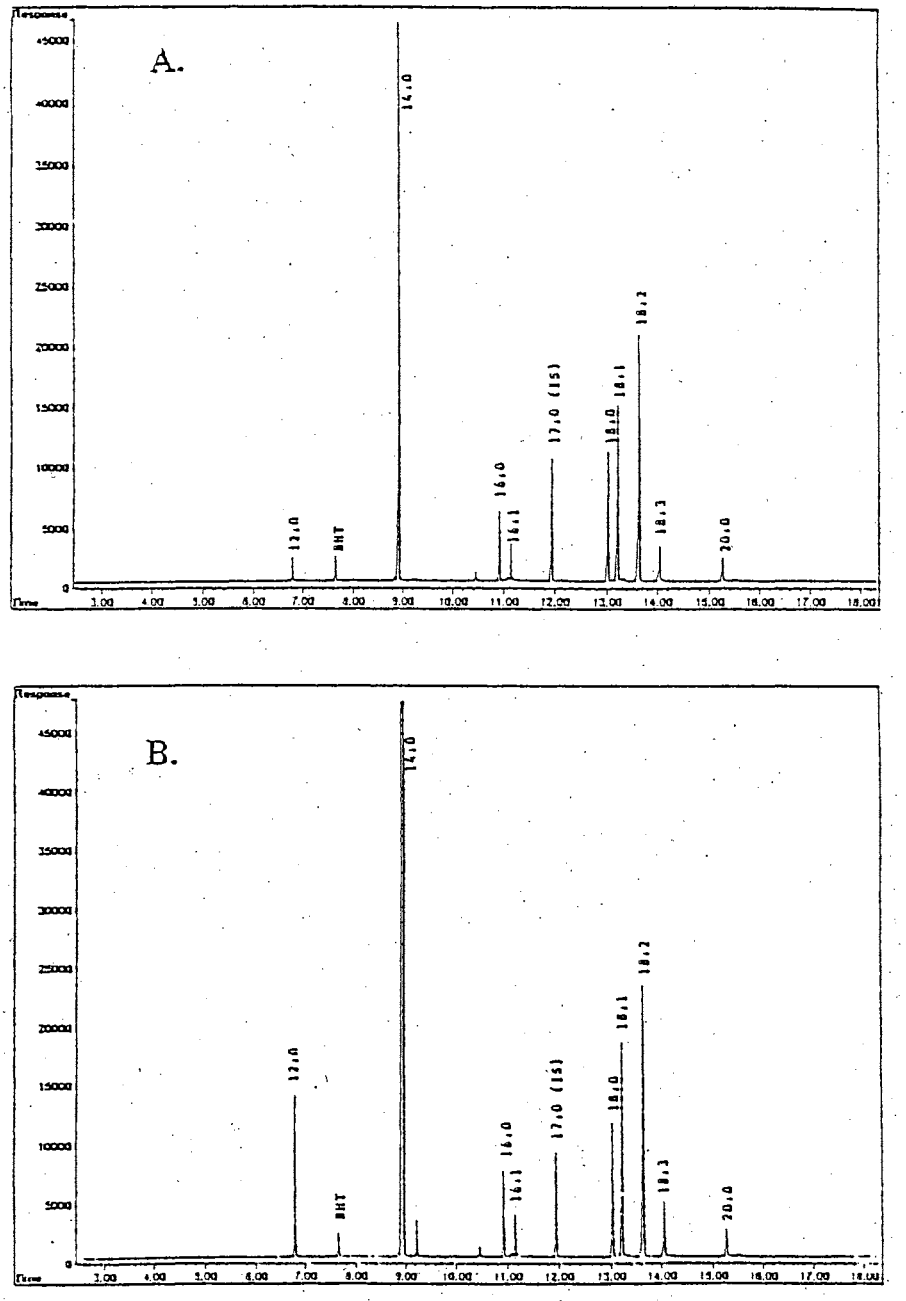

Fig. 4 GC trace of fatty acids from 25°C (A) and 10°C (B).

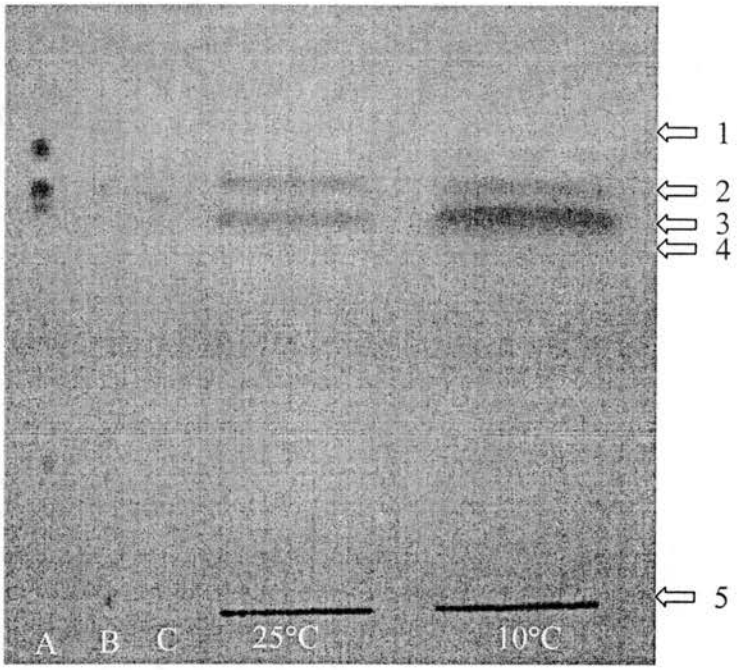

Fig. 5 Separation of total fatty acid in pea aphids by thin layer chromatography. A: Sigma 178-1 standard ; B. Sigma 178-3 standard; C. Trimyristin standard. 1. Cholesterol ester; 2. Triglyceride I; 3. Triglyceride II; 4. Triglyceride III; 5. Phospholipids.

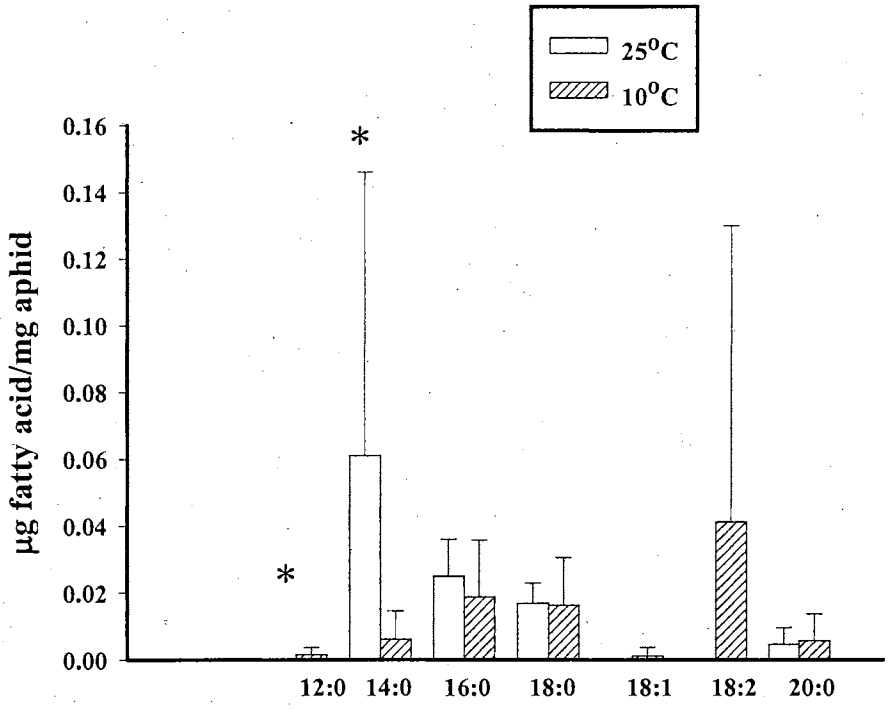

Fig. 6 Fatty acid profiles of cholesterol ester in pea aphids reared at two colonies. (*) indicates significant differences, based on $p \leq 0.05$, student's *t* test. Bars represent mean ± standard deviation (n=3).

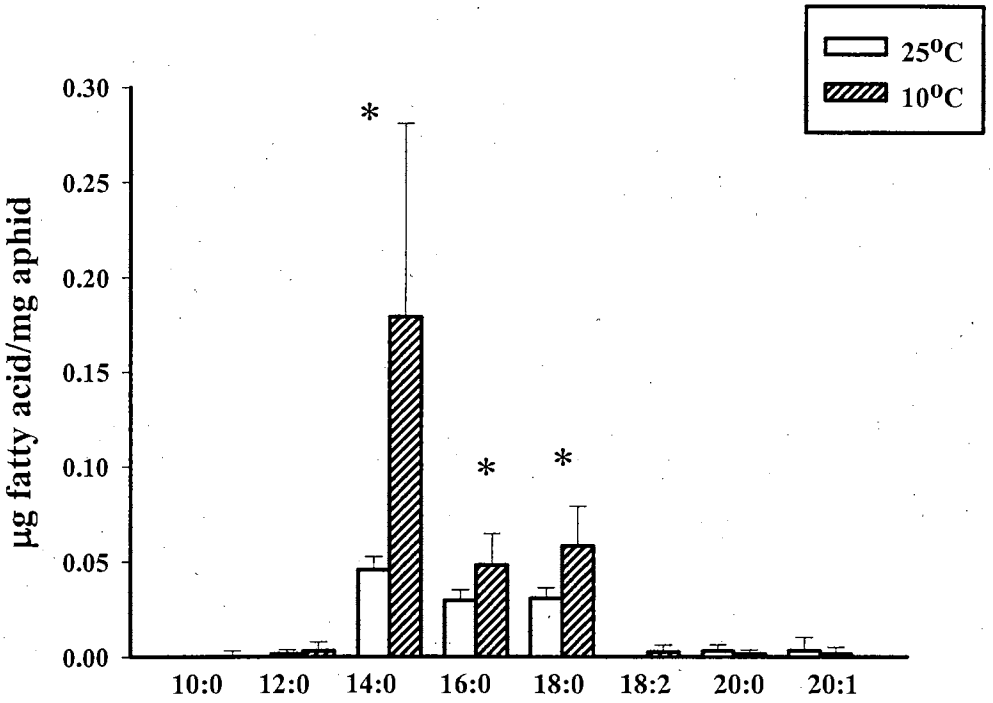

Fig. 7 Fatty acid profiles of triglyceride I in pea aphids reared at two colonies. (*) indicates significant differences, based on $p \leq 0.05$, student's *t* test. Bars represent mean ± standard deviation (n=3).

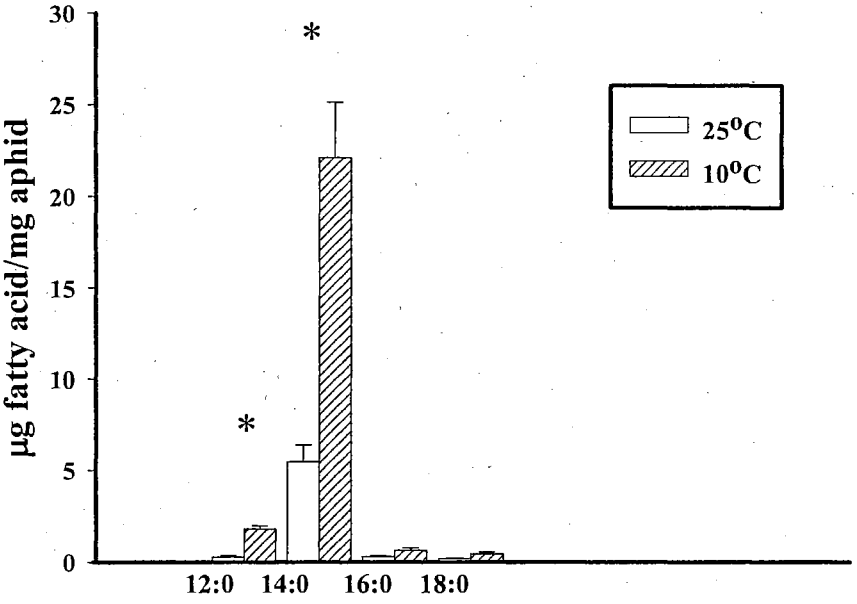

Fig. 8 Fatty acid profiles of triglyceride II in pea aphids reared at two colonies. (*) indicates significant differences, based on $p \leq 0.05$, student's *t* test. Bars represent mean ± standard deviation (n=3).

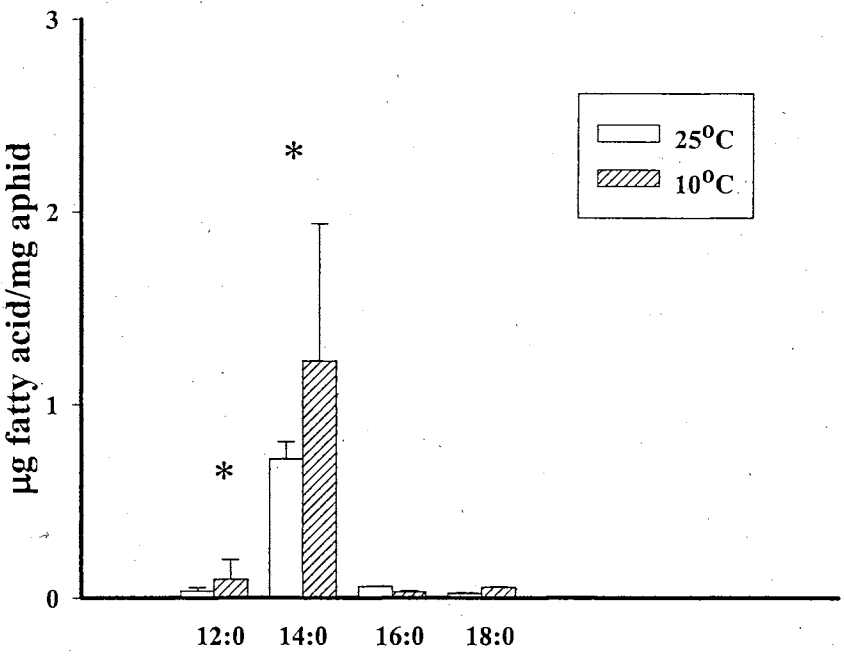

Fig. 9 Fatty acid profiles of triglyceride III in pea aphids reared at two colonies. (*) indicates significant differences, based on $p \leq 0.05$, student's *t* test. Bars represent mean ± standard deviation (n=3).

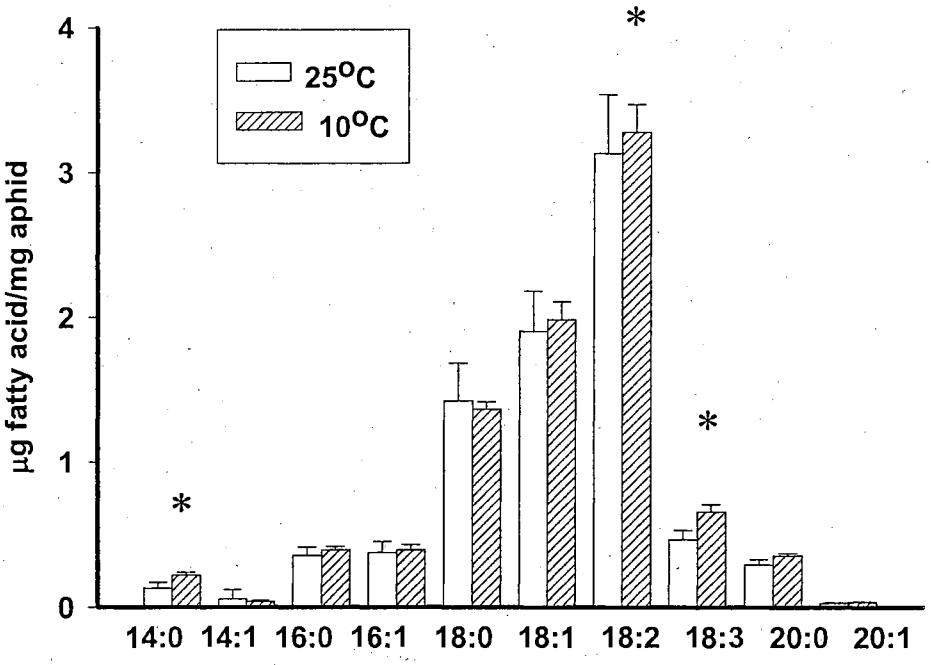

Fig. 10 Fatty acid profiles of phospholipids in pea aphids reared at two colonies. (*) indicates significant differences, based on $p \leq 0.05$, student's *t* test. Bars represent mean ± standard deviation (n=3).

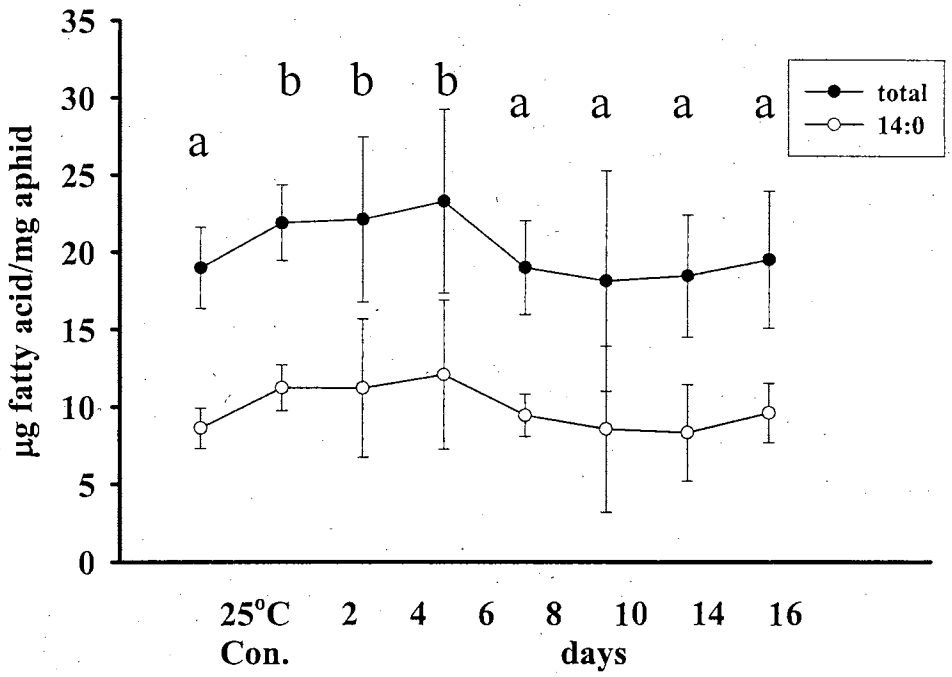

Fig. 11 Levels of total and myristic acid in pea aphids reared at 20°C. The different letters indicate significant difference, based on $p \leq 0.05$, student's *t* test. Bars represent mean ± standard deviation (n=3).

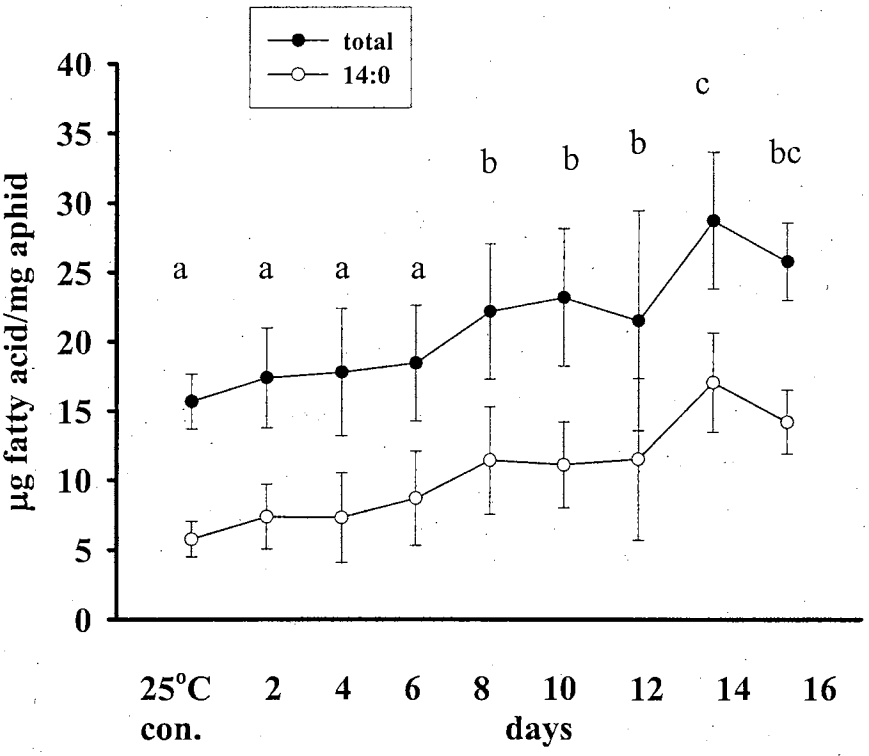

Fig. 12 Levels of total and myristic acid in pea aphids reared at 15°C (treatment 1). The different letters indicate significant differences, based on $p \leq 0.05$, student's *t* test. Bars represent mean ± standard deviation (n=3).

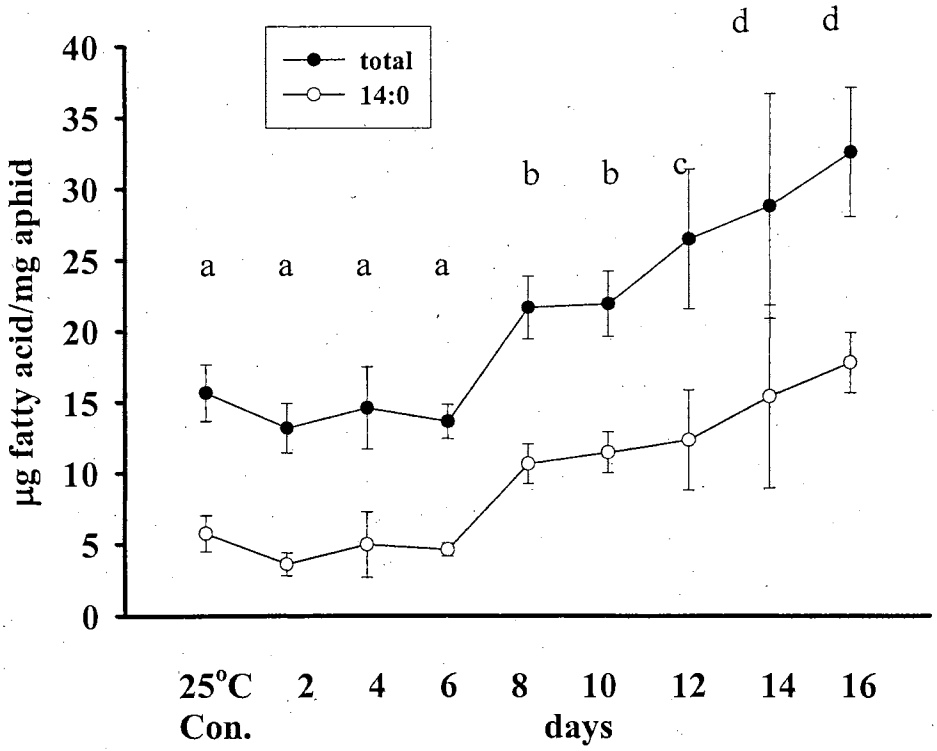

Fig. 13 Levels of total and myristic acid in pea aphids reared at 10°C. The aphids were infested from 25°C colony. The different letters indicate significant differences, based on $p \leq 0.05$, student's *t* test. Bars represent mean ± standard deviation (n=3).

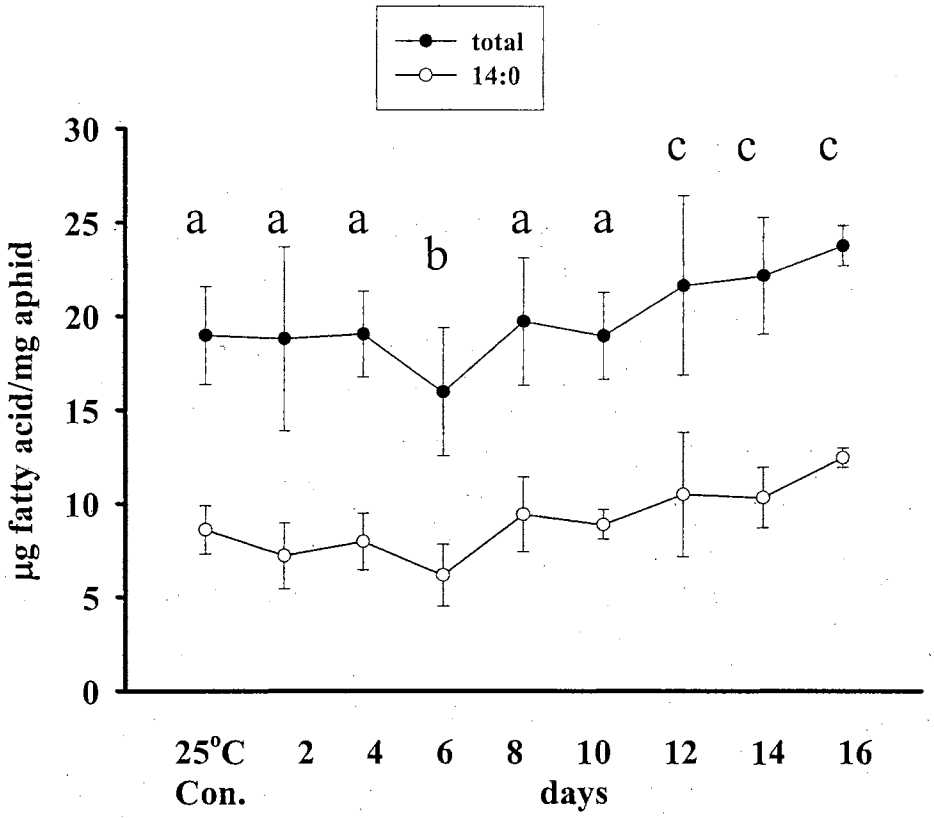

Fig. 14 Levels of total and myristic acid in pea aphids reared at 5°C. The different letters indicate significant differences, based on $p \leq 0.05$, student's *t* test. Bars represent mean ± standard deviation (n=3).

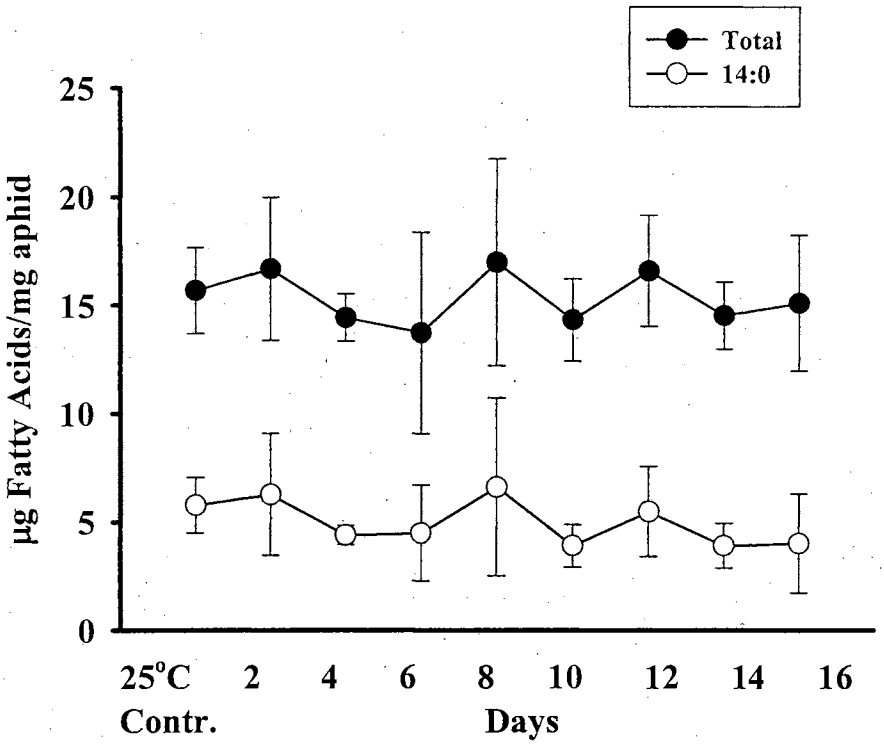

Fig. 15 Levels of total and myristic acid in pea aphids reared at 4°C. The aphids were infested from 25°C colony. They were not statistical differences compared to control (n=3).

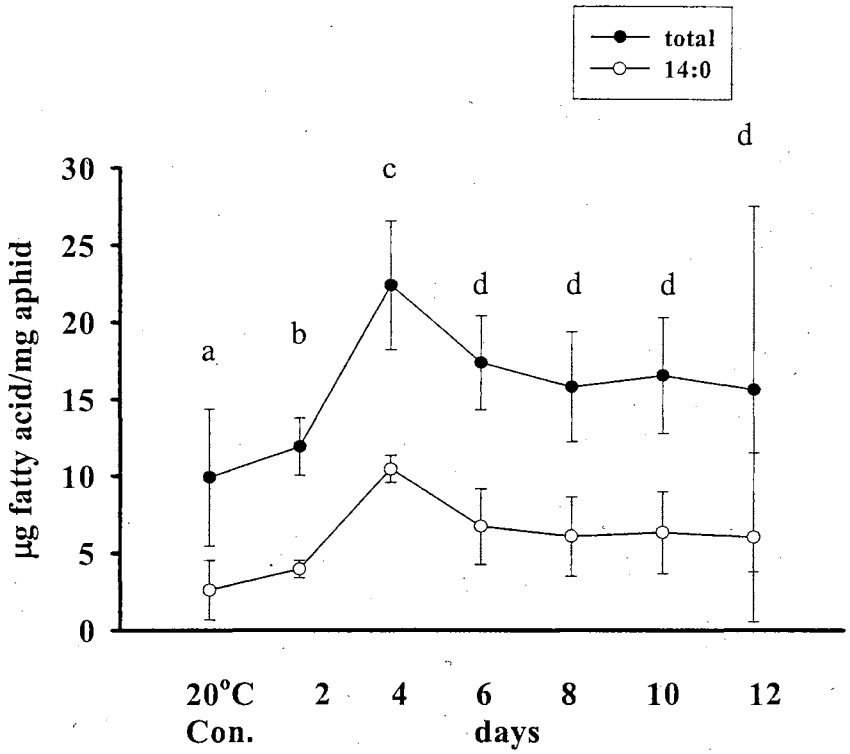

Fig. 16 Levels of total and myristic acid in pea aphids reared at 15°C. The aphids were infested from 20°C aphids. The different letters indicate significant differences, based on $p \leq 0.05$, Student's *t* test. Bars represent mean ± standard deviation (n=3).

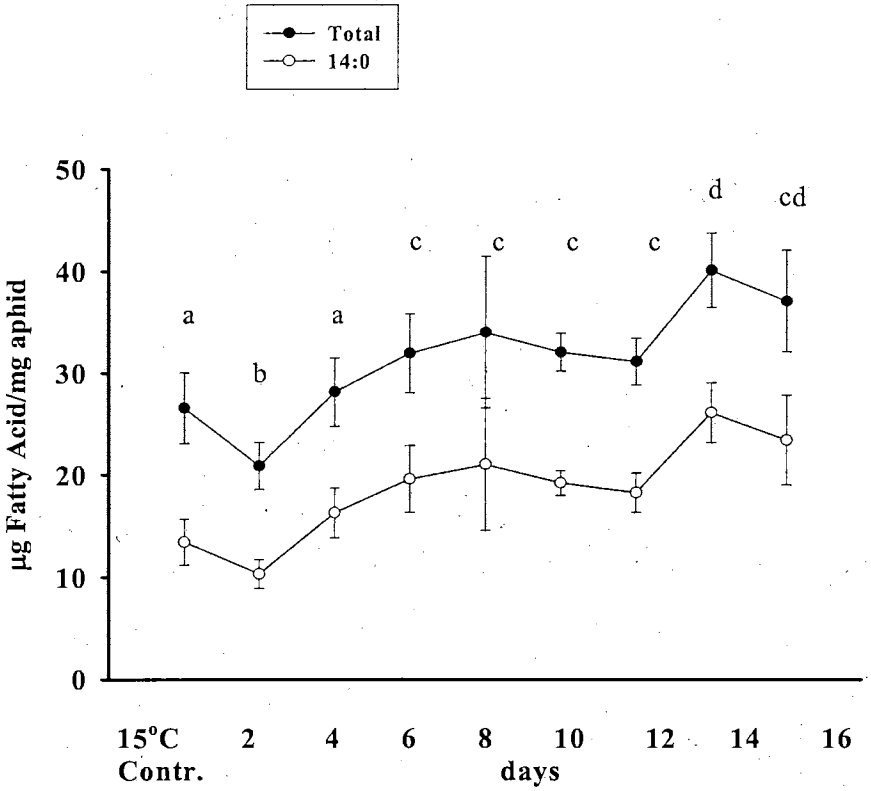

Fig. 17 Levels of total and myristic acid in pea aphids reared at 10°C. The aphids were infested from 15°C aphids. The different letters indicate significant differences, based on $p \leq 0.05$, student's *t* test. Bars represent mean ± standard deviation (n=3).

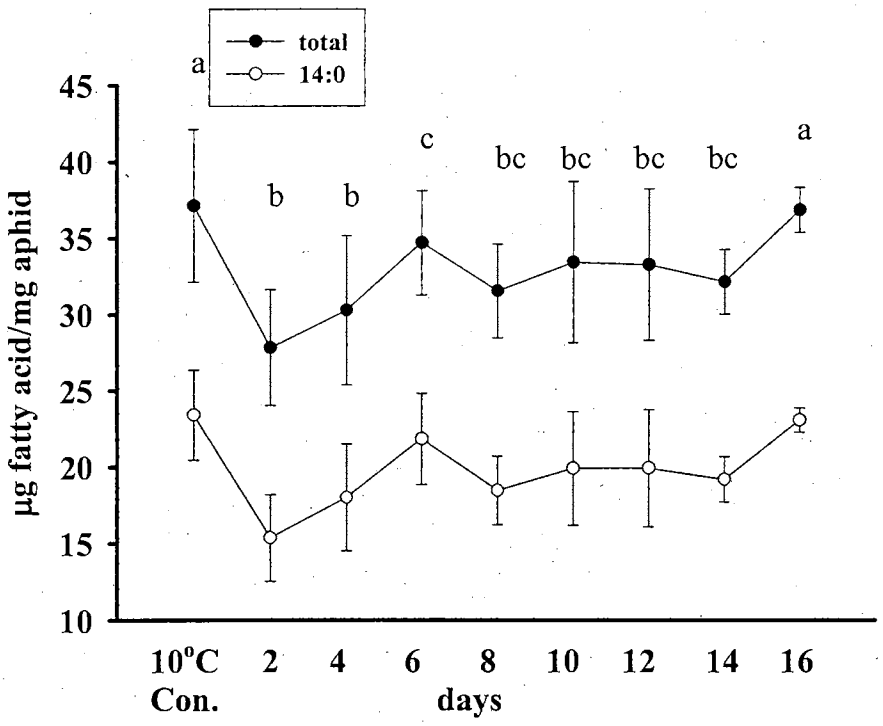

Fig. 18 Levels of total and myristic acid in pea aphids reared at 5°C. The aphids were infested from 10°C colony. The different letters indicate significant differences, based on $p \leq 0.05$, student's *t* test. Bars represent mean ± standard deviation (n=3).

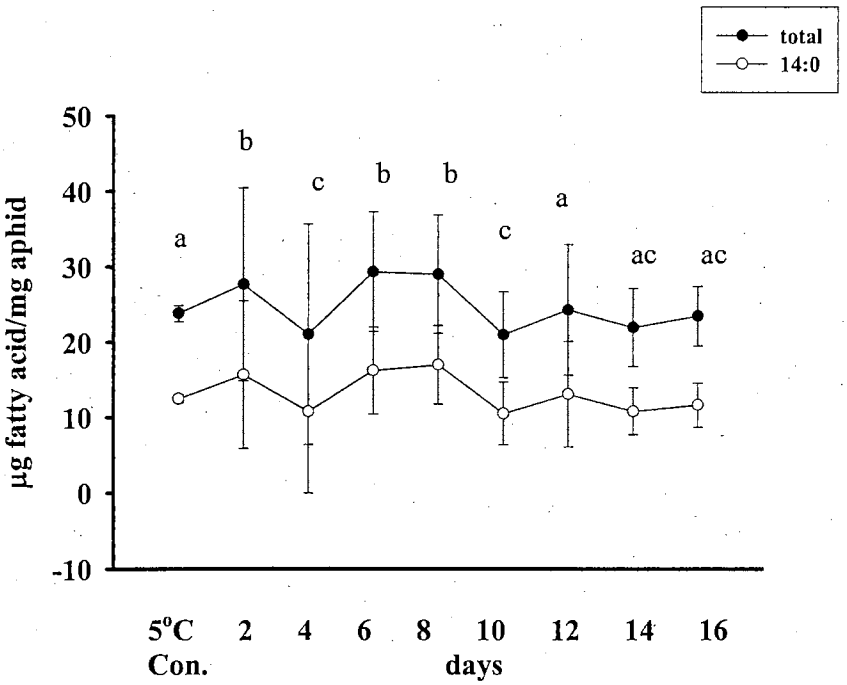

Fig. 19 Levels of total and myristic acid in pea aphids reared at 4°C. The aphids were infested from 5°C colony. The different letters indicate significantly different, based on $p \leq 0.05$, student's *t* test. Bars represent mean ± standard deviation (n=3).


tn1 1 MPPQGQTG----------GSWVLYETDAVNTDT----DAP--VIVPPSAEKREWKIVWRNVILMGMLHIGGV 56
on1 1 MAPNIKDGA----------DLNGVLFEDDASTPDY----ALATAPVQKADNYPR--KLVWRNIILFAYLHLAAV 58
hz2 1 MAPNISED----------VNGVLFESDAATPDL----ALSTPPVQKADNRPK--QLVWRNILLFAYLHLAAL 56
hz1 1 MPPQGQTG----------GSWVLYETDAVNEDT----DAP--VIVPPSAEKREWKIVWRNVILMGMLHIGGV 56
dm6 1 MAPYSRIYHQDKSS----------RE--TGVLFEDDAQTVDG----DLTTDRFQLKRAEKRRLPLVWRNIILFALVHLAAL 65
dm5 1 MAPYSRIYHQYKSS----------RE--TGVLFEDDAQTVDG----DLTTDRFQLKRAEKRRLPLVWRNIILFALVHLAAL 65
dm4 1 MAPYSRIYHQDKSS----------RE--TGVLFEDDAQTVDS----DLTTDRFQLKRAEKRRLPLVWRNIILFALVHLAAL 65
dm3 1 MPPNAQAGAQSISDSLIAAASAAADAGQSPTKLQEDSTGVLFECDVETTDG----GLVKDITVMKKAEKRLLKLVWRNIIAFGYLHLAAL 86
dm2 1 MPPNAQAGAQSISDSLIAAASAAADAGQSPTKLQEDSTGVLFESDVETTDG----GLVKDITVMKKAEKRRLKLVWRNIIVFGYFHLAAL 86
dm10 1 MAPYSRIYHQDKSS----------RE--TGVLFEDDAQTVDG----DLTTDRFQLKRAEKRRLPLVWRNIILFALVHLAAL 65
dm1 1 MPPNAQAGAQSISDSLIAAASAAADAGQSPTKLQEDSTGVLFECDVETTDG----GLVKDITVMKKAEKRRLKLVWRNIIAFGYLHLAAL 86
bm1 1 MPPQRKQE----------ASWVLYEADANNLPE----DAP--PHVPPSAEKRPWKIVWRNVILFFILHVGGV 56
av1 1 MAPNVTE----------NGVLFESDAATPDL----ALGTAPVEQADDSPR--VYVWRNIILFAYLHLAAI 54
ad1 1 MAPNITSAP----------TGVLFEGDTIGPAAKDQQAEVNAPEAKKPREPYRRQIVWRNVILFIYLHLAAL 62
aa1 1 MSTVTETVTEER----------PAS---KPHKMEIVWRNVILMGSLHLISI 38
tn1 57 YGAYLFLTKAMWLTDLFAFFLYLCSGLGITAGAHRLWAHKSYKARLPLRLLLTLFNTLAFQDAVIDWARDHRMHHKYSETDADPHNATRG 146
on1 59 YGAYLFLFSAKWQTDIFAYILYVISGLGITAGAHRLWAHKSYKAKWPLRLILIIFNTVSFQDSALDWSRDHRMHHKYSETDADPHNATRG 148
hz2 57 YGGYLFLFSAKWQTDIFAYILYVISGLGITAGAHRLWAHKSYKAKWPLRVILVIFNTVAFQDAAMDWARDHRMHHKYSETDADPHNATRG 146
hz1 57 YGAYLFLTTAMWRTCIFAVVLYICSGLGITAGAHRLWAHKSYKARLPLRLMLTLFNTLAFQDAVIDWARDHRMHHKYSETDADPHNATRG 146
dm6 66 YGLHSIFTRAKLATTLFAAGLYIIGMLGVTAGAHRLWAHRTYKAKWPLRLLLVIFNTIAFQDAVYHWARDHRVHHKYSETDADPHNATRG 155
dm5 66 YGLHSIFTRAKLATTLFAAGLYIIGMLGVTAGAHRLWAHRTYKAKWPLRLLLVIFNTIAFQDAVYHWARDHRVHHKYSETDADPHNATRG 155
dm4 66 YGLHSIFTRAKLATTLFAAGLYIIGMLGVTAGAHRLWAHRTYKAKWPLRLLLVIFNTIAFQDAVYHWARDHRVHHKYSETDADPHNATRG 155
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dm2 87 YGAYLMVTSAKWQTCILAYFLYVISGLGITAGAHRLWAHRSYKAKWPLRVILVIFNTIAFQDAAYHWARDHRVHHKYSETDADPHNATRG 176
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dm1 87 YGAYLMVTSAKWQTCILAYFLYVISGLGITAGAHRLWAHRSYKAKWPLRVILVIFNTIAFQDAAYHWARDHRVHHKYSETDADPHNATRG 176
bm1 57 YGGYLFLFKAMWRTSIFAIFLYLCSGLGITAGAHRLWAHKSYKARLPLRILLTIFNTIAFQDAVVDWARDHRMHHKYSETDADPHNATRG 146
av1 55 YGGYLFLFSAKWQTDIFAYFLYVASGLGITAGAHRLWAHKSYKAKWPLRVILTTFNTIAFQDSAIDWARDHRMHHKYSETDADPHNATRG 144
ad1 63 YGAYLAFTSAKIATTIFAIILYQVSGVGITGGAHRLWAHRSYKAKWPLRVILMLCNTLAFQNHIYEWARDHRVHHKFSETDADPHNATRG 152
aa1 39 YGFYLIFFAAQWKTVLAAYIFYTISGIGVTAGSHRLWSHRSYKAKLPYRIMLMIFQTMAFQNDIYDWARDHRMHHKFSETTADPHDATRG 128
Δ9F1
Δ9F2

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tn1   147 FFFSHVGWLLVRKHPQIKAKGHTIDLSDLKSDPILRFQKKYYLTLMPLICFILPSYIPT-LWGESAFNAFFVCSIFRYVYVLNVTWLVNS 235
on1   149 FFFSHIGWLLVRKHPELKRKGKGLDLSDLYADPILRFQKKYYLLLMPLGCFIMPTVVPVYFWGETWTNAFFVAALFRYTFILNVTWLVNS 238
hz2   147 FFFSHIGWLLVRKHPDLKEKGKGLDMSDLLADPILRFQKKYYLILMPLACFVMPTVIPVYFWGETWTNAFFVAAMFRYAFILNVTWLVNS 236
hz1   147 FFFAHVGWLLVRKHPQIKAKGHTIDLSDLKSDPILRFQKKYYLFLMPLVCFILPCYIPT-LWGESLWNAYFVCSIFRYVYVLNVTWLVNS 235
dm6   156 FFFSHVGWLLCKKHPDIKEKGRGLDLSDLRADPILMFQRKHYYILMPLACFVLPTVIPMVYWNETLASSWFVATMFRWCFQLNMTWLVNS 245
dm5   156 FFFSHVGWLLCKKHPDIKEKGRGLDLSDLRADPILMFQRKHYYILMPLACFVLPTVIPMVYWNETLASSWFVATMFRWCFQLNMTWLVNS 245
dm4   156 FFFSHVGWLLCKKHPDIKEKGRGLDLSDLRADPILMFQRKHYYILMPLACFVLPTVIPMVYWNETLASSWFVATMFRWCFQLNMTWLVNS 245
dm3   177 FFFSHVGWLLCKKHPEVKAKGKGVDLSDLRADPILMFQKKYYMILMPIACFIIPTVVPMYAWGESFMNAWFVATMFRWCFILNVTWLVNS 266
dm2   177 FFFSHVGWLLCKKHPEVKAKGKGVDLSDLRADPILMFQKKYYMILMPIACFIIPTVVPMYAWGESFMNAWFVATMFRWCFILNVTWLVNS 266
dm10  156 FFFSHVGWLLCKKHPDIKEKGRGLDLSDLRADPILMFQRKHYYILMPLACFVLPTVIPMVYWNETLASSWFVATMFRWCFQLNMTWLVNS 245
dm1   177 FFFSHVGWLLCKKHPEVKAKGKGVDLSDLRADPILMFQKKYYMILMPIACFIIPTVVPMYAWGESFMNAWFVATMFRWCFILNVTWLVNS 266
bm1   147 FFFSHIGWLLLRKHPEIKAKGHTVDVNELRNDPILRFQKKYYQILMPLACFIMPTYVPT-LWGETVWNSFYVCAIFRYVYVLNITWLVNS 235
av1   145 FFFSHIGWLLVRKHPELKRKGKGLDLSDLYADPILRFQKKYYLLLMPLACFILPTVIPVYLWNETWTNAFFVAALFRYTFILNVTWLVNS 234
ad1   153 FFFSHVGWLLVRKHPDVKEKGKGIDMHDLEQDKIVMFQKKYYLILMPIVCFLIPTTIPVYMWNETWSNAWFVATLFRYTFTLNMTWLVNS 242
aa1   129 FFFSHVGWLLVRKHPDVRNKGKSIDLSDVLADPVVRFQRRYYLPLMVTICFIVPALLPWWLWGETLWNSFVVCSLTRYCFTLNMTWLVNS 218
          ***.*.**** .*** .. **. .*. ..  * .. **.. *  **   **..*. .*   *.*.   .  *... *  . **.******
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tn1   236 AAHLWGSKPYDKNINPVETRPVSLVVLGEGFHNYHHTFPWDYKTAELGDYSLNFTKMFIDFMASIGWAYDLKTVSTDVIQKRVKRTGDGS 325
on1   239 AAHKWGHKPYDSSIKPSENLSVSLFALGEGFHNYHHTFPWDYKTAELGNNRLNFTTNFINFFAKIGWAYDLKTVSDEIIQNRVKRTGDGS 328
hz2   237 AAHKWGDKPYDKSIKPSENLSVAMFALGEGFHNYHHTFPWDYKTAELGNNKLNFTTTFINFFAKIGWAYDLKTVSDDIVKNRVKRTGDGS 326
hz1   236 AAHLWGAKPYDKNINPVETRPVSLVVLGEGFHNYHHTFPWDYKTAELGDYSLNLTKLFIDTMAAIGWAYDLKTVSTDVIQKRVKRTGDGS 325
dm6   246 AAHKFGNRPYDKTMNPTQNAFVSAFTFGEGWHNYHHAFPWDYKTAEWGCYSLNITTAFIDLFAKIGWAYDLKTVAPDVIQRRVLRTGDGS 335
dm5   246 AAHKFGNRPYDKTMNPTQNAFVSAFTFGEGWHNYHHAFPWDYKTAEWGCYSLNITTAFIDLFAKIGWAYDLKTVAPDVIQRRVLRTGDGS 335
dm4   246 AAHKFGNRPYDKTMNPTQNAFVSAFTFGEGWHNYHHAFPWDYKTAEWGCYSLNITTAFIDLFAKIGWAYDLKTVAPDVIQRRVLRTGDGS 335
dm3   267 AAHKFGGRPYDKFINPSENISVAILAFGEGWHNYHHVFPWDYKTAEFGKYSLNFTTAFIDFFAKIGWAYDLKTVSTDIIKKRVKRTGDGT 356
dm2   267 AAHKFGGRPYDKFINPSENISVAILAFGEGWHNYHHVFPWDYKTAEFGKYSLNFTTAFIDFFAKIGWAYDLKTVSTDIIKKRVKRTGDGT 356
dm10  246 AAHKFGNRPYDKTMNPTQNAFVSAFTFGEGWHNYHHAFPWDYKTAEWGCYSLNITTAFIDLFAKIGWAYDLKTVAPDVIQRRVLRTGDGS 335
dm1   267 AAHKFGGRPYDKFINPSENISVAILAFGEGWHNYHHVFPWDYKTAEFGKYSLNFTTAFIDFFAKIGWAYDLKTVSTDIIKKRVKRTGDGT 356
bm1   236 AAHMWGSKPYDKNINPVETRPVSLVVLGEGFHNYHHTFPWDYKTAELGDYSLNLSKLFIDFMAKIDWAYDLKTVSTDVIQKRTKRTGDGS 325
av1   235 AAHKWGDKPYDKSIKPSENMSVSLFAFGEGFHNYHHTFPWDYKTAELGNHRLNFTTKFINFFAKIGWAYDMKTVSNEIVQQRVKRTGDGS 324
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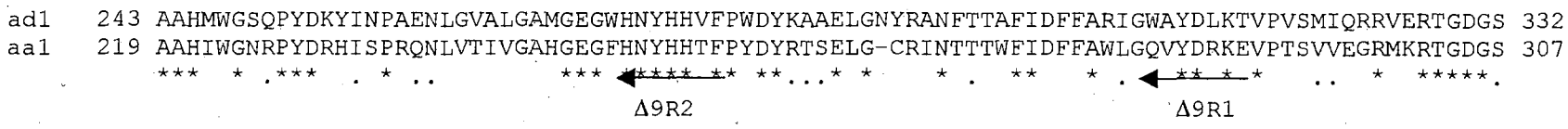


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on1   329 HHLWGWGDKDQPKEE-VNAAIRINPKDE 355
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hz1   326 HPVWGWDDHEVHQADKKLAAIINPEKTE 353
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dm5   336 HELWGWGDKDLTAED-ARNVLLVDKSR  361
dm4   336 HELWGWGDKDLTAED-ARNVLLVDKSR  361
dm3   357 HATWGWGDVDQPKEE-IEDAVITHKKSE 383
dm2   357 HATWGWGDVDQPKEE-IEDAVITHKKSE 383
dm10  336 HELWGWGDKDLTAED-ARNVLLVDKSR  361
dm1   357 HATWGWGDVDQPKEE-IEDAVITHKKSE 383
bm1   326 HPVWGYDVGEVATEDKTDTTNLVNSKVL 353
av1   325 HHLWGWGDKDHAQEE-INAAIRIHPKDD 351
ad1   333 HEVWGWGDKDMPQED-IDGAVIEKRKTQ 359
aa1   308 RGLTAGTRSW                   317
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Fig. 20 Sequence alignment and primer design. Two pairs of primers, Δ9F1/R1 and Δ9F2/R2 were designed based on the sequence alignment (indicated as arrows in the figure). aa: *Amblyomma americanum*; ad: *Acheta domestica*; av: *Argyrotaenia velutinana*; bm: *Bombyx mori*; dm: *Drosophila melanoguster*; hz: *Helicoverpa zea*; on: *Ostrinia nubilalis*; tn: *Trichoplusia ni*.

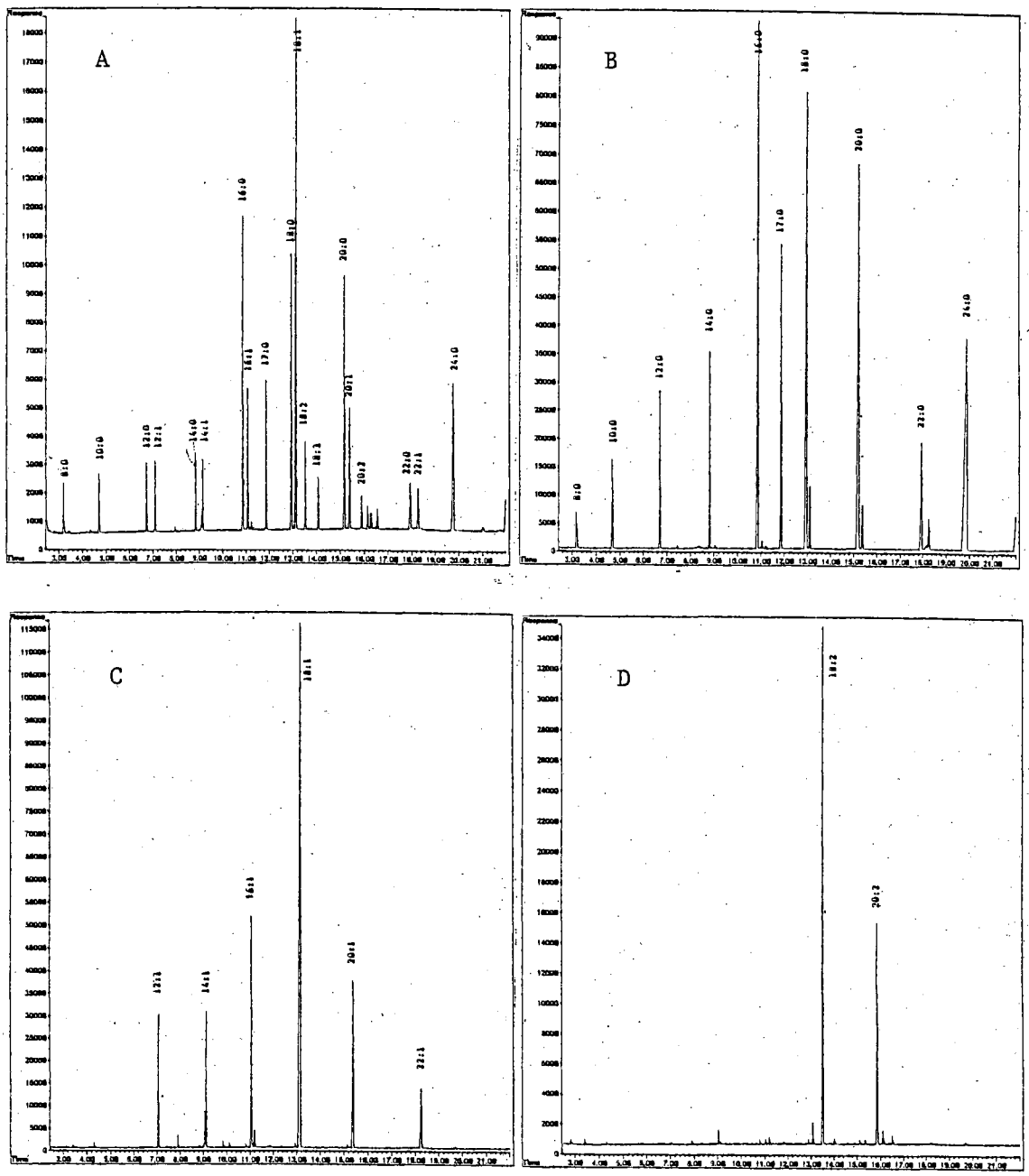

Fig. 21 Separation of fatty acid standard by silver ion chromatography and detected by GC. A. Profile of fatty acid standard; B. Saturated fatty acids; C. Monoenes; D. Dienes.

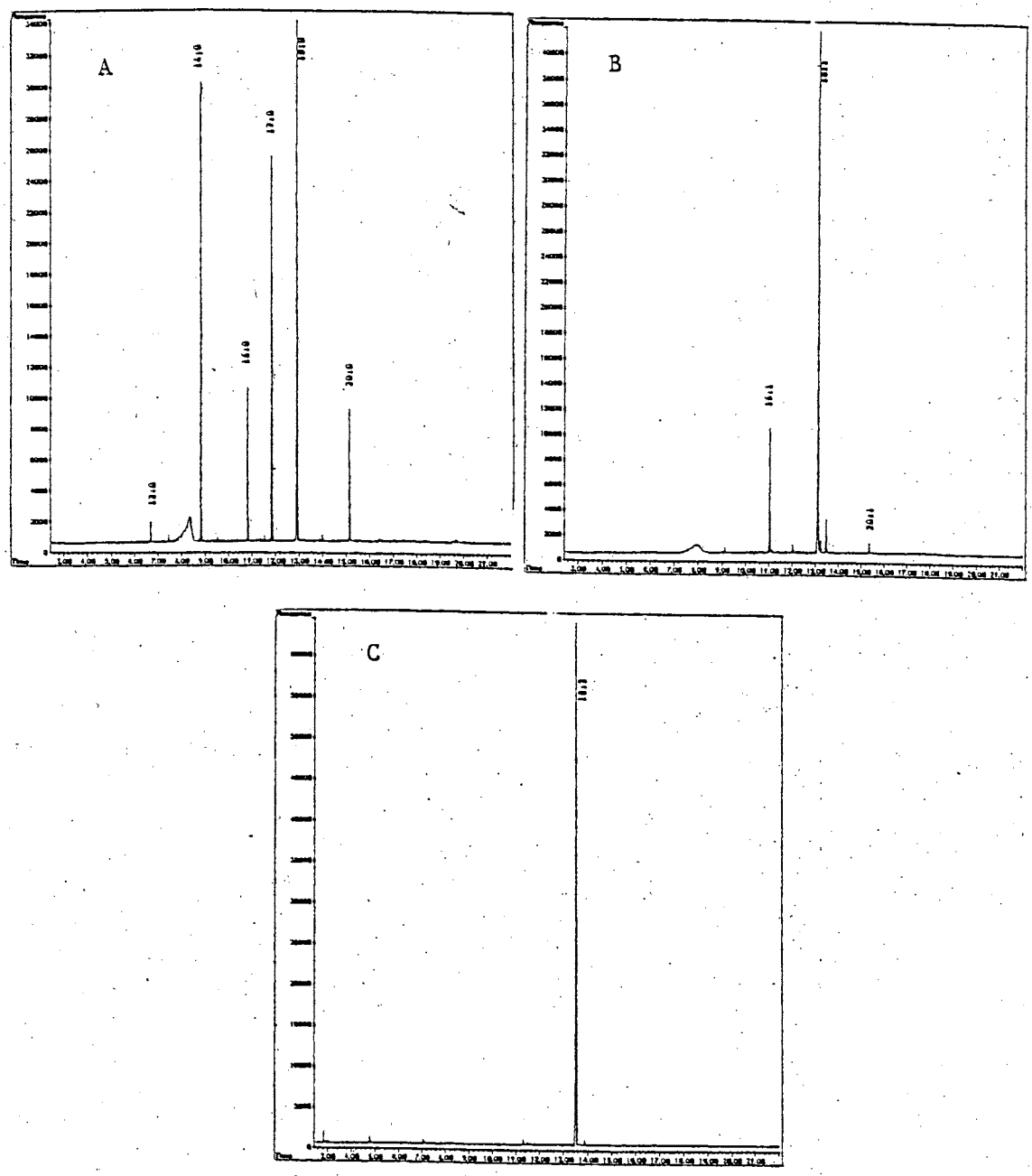

Fig. 22 Separation of fatty acids from microsomal in the pea aphid by silver ion chromatography and detected by GC. A. Saturated fatty acids; B. Monoenes; C. Dienes.

VITA

Zhaorigetu Chen 2

Candidate for the Degree of

Doctor of Philosophy

Dissertation: INFLUENCE OF REARING TEMPERATURE ON FATTY ACID METABOLISM IN THE PEA APHID, *Acyrthosiphon pisum*

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