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FLUORANTHENE AFFECTS DIGESTIVE ENZYME ACTIVITY AND RELATIVE DEVELOPMENT RATE OF LYMANTRIA DISPAR L. LARVAE

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Abstract

Among the most frequent polycyclic aromatic hydrocarbon contaminants in the environment, fluoranthene may accumulate in plant leaves, which are the primary food source for phytophagous insect species. Fluoranthene is a carcinogen that can cause cancer. Fluoranthene is a carcinogen that can build in the environment. Fluoranthene is a carcinogen that has been linked to the development of cancer. To find out what happens when you feed fluoranthene to larvae of Lymantriadispar and Euproctischrysorrhoea, we conducted a study to find out what happens to their digestive enzyme specific activity and expression of their isoforms in the midgut of these two species' larvae, as well as what happens to their relative growth rates. Upon exposure to fluoranthene, trypsin activity in the midguts of larvae of both species was shown to have decreased significantly when the chemical was used. After being exposed to a lower concentration of fluoranthene, the activity of the enzyme leucine aminopeptidase decreased significantly in the midguts of L. dispar larvae, whereas the same enzyme activity increased significantly in the midguts of E. chrysorrhaea larvae when exposed to a higher concentration of fluoranthene. It was discovered that exposure to pollutants did not affect the lipase activity of L. dispar, but that it did enhance the enzyme activity of E. coli's midgut, which was previously unobserved.

Keywords: Fluoranthene,LeucineAminopeptidase, Polycyclic Aromatic, Cancer

INTRODUCTION

A family of complex chemical compounds known as polycyclic aromatic hydrocarbons (PAHs) is composed mostly of two or more fused benzene rings, which are created when two or more benzene rings unite. Despite the fact that there are natural sources of PAHs, such as volcanic activity and forest fires, the vast majority of PAHs found in the environment are a result of human activities, such as industrial production and agriculture (Srogi, 2007). "PAHs may be found in large amounts in the environment," says the researcher. As a result of their environmental durability, inclination for bioaccumulation, and a range of negative consequences on both people and the environment, these pollutants are a source of substantial public concern." Fluoranthene is a four-ringed polycyclic aromatic hydrocarbon (PAH) that has been identified by the United States Environmental Protection Agency as one of the 16 priority pollutant polycyclic aromatic hydrocarbons (PAHs). Fluoranthene is a carcinogenic and toxic polycyclic aromatic hydrocarbon (PAH) that has been identified as one of the 16 priority pollutant polycyclic aromatic hydrocarbons (PAHs) (Saunders et al., 2003). According to the type of plant, phytochemicals (PAHs) may reach plants via the atmosphere or contaminated soil, where they can be absorbed by plant shoots or roots, depending on the source of the PAH. "PAH transfer from the air to the leaves, either by deposition on the leaf cuticle or absorption through stomata, is now believed to be the most important way of contamination for plants growing on unpolluted

soils, according to currently accepted thinking. When it comes to the absorption and accumulation of lipophilic PAH compounds in leaves, a range of leaf qualities, such as surface roughness and hair presence, as well as other leaf components, such as waxes and lipids, are important factors to consider (Desalme et al., 2013)." High-level plants have been demonstrated to be negatively affected by the chemical fluoranthene. In particular, phytophagous species may be significantly harmed by this development.

Proteinases are important enzymes in the insect digestive system because they catalyse the breakdown of peptide bonds, which is necessary for digestion to take place. Proteases that hydrolyze internal peptide bonds of the polypeptide chain on the carboxyl side of basic L-amino acids, such as trypsin, are responsible for the majority of the protease activity seen in the midgut of Lepidoptera. They are responsible for as much as 95% of the total digestive action in the human body (Terra and Ferreira, 1994; Srinivasan et al., 2006). As the name implies, aminopeptidases are metalloenzymes that are responsible for detaching amino acid residues from the N-terminus of polypeptide chains at the "N-terminus. Insect midgutaminopeptidases have been proposed to have a key role in the intermediate stage of protein digestion, according to recent research (Lomate&Hivrale, 2010). They do this by hydrolyzing oligopeptides that have been partially digested."

It is a kind of enzyme that catalyses the breakdown of ingested lipids, therefore supplying larvae with the fatty acids they need for development. Lipases are found in both plants and animals. Isozymes are enzymes that exist in a variety of molecular forms that are distinct from one another. They are classified as follows: The fact that they both have the same catalytic activity despite the fact that they are structurally different allows for more flexibility, adaptability, and precision while conducting specific metabolic activities. The impacts of plant allelochemicals, as well as pesticides and heavy metals, which are all significant environmental contaminants, have previously been studied in some detail. Per Charron and colleagues (2013), PAHs are most often discovered in the urine of aquatic species whose ecosystems have been considerably affected by human activity (as in Vignet et al., 2014). The fact that there is evidence that PAHs have an effect on the enzyme activities of other species notwithstanding, to the best of our knowledge, research into the influence of PAHs on the digestive enzymes of phytophagous insects are still in their early stages. A large number of studies have shown that PAHs have an impact on the fitness of phytophagous insects (Mrdakovi c et al., 2015). A considerable percentage of an insect's energy resources are diverted away from development and toward metabolism and defence in order to offset the negative effects of a pollutant, resulting in reduced growth and reproduction for the insect. In this way, the affects on an individual's biochemical level and the implications on the community as a whole are linked together via fitness-related features (Hyne and Maher, 2003). It is possible that changes in fitness characteristics may provide significant information on the potential effects of pollutants on a certain insect species' population, and also on how those insects will adapt to those contaminants.

In the present study, we are building on our previous findings on the coping methods of insects that have been exposed to a pollutant in the environment. The activity of "antioxidative and phase II biotransformation enzymes in L. dispar and E. chrysorrhoea larvae that have been exposed to dietary fluorantheneover a prolonged length of time has previously been shown in our laboratory. In this investigation, we looked into the effects of dietary fluo- ranthene at ecologically relevant amounts on the function of many digestive enzymes (trypsin, leucine aminopeptidase, and lipase)."

MATERIALS AND METHODS

Insect rearing and fluoranthene treatments

L. dispar egg masses and E. chrysorrhoea winter nests were collected in November from a mixed oak forest near the city of Majdanpek (East Serbia). Winter nests of E. chrysorrhoea were also kept at 4 degrees Celsius until late March, when they were transported to room temperature and placed on wild plum trees that had buds on the branches of the trees. A temperature of 26 0.5 degrees Celsius was maintained throughout the experiment, as well as a photoperiod of 16 hours and eight minutes. It was discovered that the larvae of each species were randomly assigned to one of three groups and fed an artificial diet with a high proportion of wheat germ (O'Dell et al., 1985). In the two experimental groups, fluoranthene was present at quantities of 6.7 ng/g dry food weight (Fl group) or 67 ng/g dry food weight (Fl group) in the form of fluoride in their diets (Fh group). The control groups (C) for both species were fed a meal that was free of fluoranthene," the researchers write. A comparison of the lower concentration of fluo- ranthene used in this study with that previously reported in the leaves of many tree species (Howsam et al., 2000), which included leaves of host plants that were suitable for both species, led to the conclusion that the lower concentration used in this study was appropriate. Researchers discovered that the increased amount corresponded to PAH levels previously identified in the leaves of other plant species, according to the research results (e.g. Alfani et al., 2001). Furthermore, when we investigated the effects of various "fluoranthene concentrations on the life history features and antioxidative enzyme activities of L. dispar larvae, we discovered that the concentrations used (6.7 and 67 ng) caused significant modification in the life history features and antioxidative enzyme activities of the larvae. In order to include fluoranthene into the false diet, it was required to dilute it in reagent-grade acetone to the appropriate concentrations." It was necessary to distribute the combination using a plastic container, which was then kept in a fume hood for 4 hours to ensure that all of the acetone had been totally evaporated. As long as the larvae did not get overfed, they were checked daily for moulting and were given equal amounts of fresh food every 48 hours as long as they did not become obese. To preserve their lives, the larvae were reared until they reached the third day of the fifth instar, at which time they were murdered.

Native electrophoresis

By electrophoresis on a 10 percent nondenaturing polyacrylamide gel (using a modified approach developed by Erlanger et al., 1961), at 100 V at 4 C, with 5 g of protein in each lane, the activity of trypsin was evaluated by the authors.Following electrophoresis, “the gel was rinsed with deionized water for 10 minutes before being soaked in 50 mM glycine buffer (pH 10) for 20 minutes to remove any remaining electrolytes. For an additional 50 minutes at room temperature, the nitrocellulose membrane was pre-treated with 2 mM substrate N-benzoylaminodlarginine 4-nitroanilide hydrochloride and incubated in the same solution for the same amount of time. One hour later, the membrane was put on the gel and allowed to sit in the wet chamber at 37 degrees Celsius.” Next, the membrane was incubated for 2.5 minutes in 0.1 percent $NaNO_2$ dissolved in 1 M HCl before being rinsed with one-tenth of a “percent urea and incubated for another 2.5 minutes in 0.05 percent 1-naphthylamine dissolved in 47.5 percent ethanol until pink bands of trypsin activity showed.”

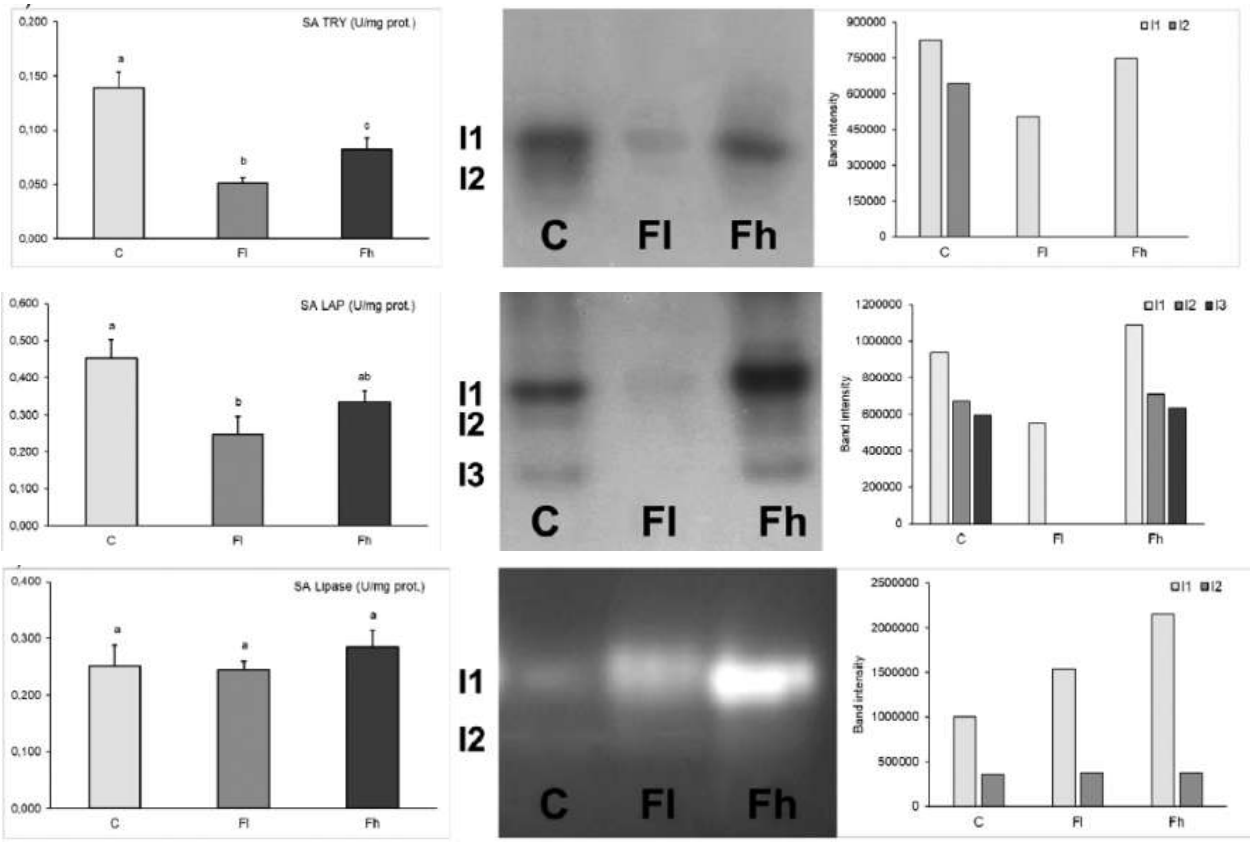

Fig. 1. Specific activities, and native polyacrylamide gels with densitometric analyses of bands intensities of: trypsin "(a), leucine aminopeptidase (b), and lipase (c) in the midgut of Lymantriadispar larvae exposed to lower (6.7 ng/g dry wt) (Fl), and higher (67 ng/g dry wt) (Fh) concentration of dietary fluoranthene. The bars represent mean values (±SE). Values marked with different letters differ significantly, ($P < 0.05$)." The numbers indicate enzymes isoforms (I).

DISCUSSION AND CONCLUSION

It was discovered in this study that the activity of digestive enzymes in the "midguts of L. dispar and E. chrysorrhoea larvae changed significantly in response to ingestion of fluoranthene at ecologically relevant concentrations" changed significantly in response to ingestion of fluoranthene at ecologically relevant concentrations. The larvae of both species devour a huge quantity of the leaves of their host plants in order to get the nutrients they need for growth and development in order to survive and reproduce. Digestion enzymes must work effectively in order for nutrients to be turned into energy and for energy resources to be made accessible to support growth and metabolism. Toxins in food have the potential to significantly limit the activity of these enzymes, and insects may react by altering their activity levels and/or synthesising less sensitive or insensitive variants of the enzyme in question, among other things. According to the current evidence, the impacts of pollutants on digestive enzymes will have an impact on the production or release of digestive enzymes (Dedourge-Geffard et al., 2013). As a result of this inquiry, researchers have become more aware of the potential relevance of these enzymes as biomarkers of toxicant exposure as a result of their findings (Lai et al., 2011). Xenobiotics may also have an impact on the growth and development of organisms due to their negative effects on the function of digestive enzymes. According to the conclusions of this research, "we noticed that long-term exposure to fluoranthene generated alterations in the specific activities of trypsin and leucineaminopeptidase in the midgut of larvae of the species L. dispar." Because of a

lower dietary fluoranthene level than predicted, both enzymes displayed the same pattern of inhibition as the control when compared to the control group. It was discovered that when trypsin was exposed to a higher fluoranthene concentration, it exhibited more sensitivity and that its activity was substantially decreased, demonstrating that it was more susceptible. In a study conducted by Lowe and colleagues, it was discovered that mussels exposed to poisons over a long length of time were toxic (1981). Dietary cells changed in form and synchronisation was broken in the presence of a mixture of PAHs, while the structure of the digestive cells' lysosomes was altered in the presence of Mitilysedulis.

Fluoranthene may have a similar detrimental effect on larval midgut epithelial cells, although it is not known whether or not this is the case. It is possible that fluoranthene does so by disrupting the structural and functional characteristics of the cells, which may result in decreased enzyme production and/or secretion on the part of the larvae in this case. As previously mentioned, fluoranthene may also directly inhibit enzyme activity by binding to and stabilising the substrate, or by inhibiting the catalytic site or amino acid residues involved in binding to and stabilising the substrate. It was observed in 2012 by Napole et al. (2012) that Aedesaegypti larvae exposed to Myracrodruonurundeuva leaf lectin, which was demonstrated to limit trypsin activity in the presence of trypsin inhibitor, were inhibited in a similar manner to what was discovered in 2012. It was also discovered that L. dispar larvae exposed to cadmium had lower trypsin and leucine aminopeptidase activities than control larvae, as well as lower Helicoverpaarmigera larval midgut trypsin activity when the larvae were fed a diet containing plant-inducible LAP (leucine aminopeptidase) activity than control larvae (Vlahovic et al., 2015). It has been shown that phytophagous insects are capable of overcoming some unfavourable effects generated by host plants as a consequence of the flexibility of digestive enzymes and the quick and effective modulation of their activity (Broadway, 1997; Lomate and Hivrale, 2011; War et al., 2013). Mrdakovic et al. (2014) reported that the trypsin activity of larvae of the genus Lepidochelysdispar reduced in response to allelochemical stress in a laboratory setting. Increased lipase activity under a stressful environment, on the other hand, has been shown to be an adaptive response in larvae of the species Lepidochelysdispar.

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