Intra and interspecific variation assessment in Psocoptera using near spectoscopy

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Abstract

Several species of Psocoptera are associated with and damage grains and other stored products, books, historical documents, and insect collections. Their small size and lack of expressive morphological variation make it a difficult group for species identification. The spectra of adult males and females of 10 psocid species from the genus Liposcelis were obtained by near infrared spectroscopy (NIRS) and analyzed. Each specimen was placed on a diffuse reflectance accessory of a NIR spectrometer to obtain the respective spectrum, using ten replicates for each species or sex. All spectra were analyzed by combined methods of multivariate analysis using the technique of crossed validation for the multivariate models. The analysis discriminated the species without significant overlapping among the species spectral patterns. The NIRS also revealed variation in the metabolomic profile of males and females; however, it is still possible to distinguish the species using only males or females or even from mixed sex samples. NIRS technique proved to be a powerful tool to discriminate species both at intra and interspecific levels based on dispersion spectral patterns of individual specimens.

Keywords: Biological systems, Liposcelididae, stored product pests, Vibrational spectroscopy.

1. Introduction

Psocoptera is a relatively small order of insects with 4400 species worldwide (Pascual-Villalobos et al., 2005; Dong et al., 2007); many of these species are associated with stored grains and other food products in many parts of the world (Turner, 1994; Nayak et al., 2003). Psocids used to be considered as only nuisance secondary pests and were often overlooked because of their small size and the presence of more damaging primary pests, especially in cereal grains. Before 1990, psocids were not considered serious pests of stored products; although in some countries such as Australia, they have become the most frequently encountered storage pest in some areas (Rees, 2003). However, their world-wide prevalence and increasing importance as pests contaminating food and agricultural commodities is now documented from all continents (Kucerova et al., 2006; Stejskal et al., 2006; Throne et al., 2006). The recent rise of psocids to prominence can be attributed to their varied response to management tactics that have been developed for coleopteran pests and the resistance of some psocopteran species to residual insecticides and phosphine (Nayak et al., 2003; Nayak, 2006). In Australia, detection of high levels of resistance to phosphine in psocids (Nayak et al., 2003) has elevated their pest status enormously and put them alongside the major coleopteran pests (Nayak et al., 2003). In addition, the importance of psocopteran pests has increased in recent years due to the failure of almost all registered grain protectants to control them (Nayak and Daglish, 2007).

Precise identification of pest species is fundamental to pest control, quality-control of food products and the settlement of legal disputes resulting from insect contamination of goods. However, it is difficult to define and to characterize insect biodiversity, especially in groups of little known insects, such as those in the order Psocoptera. A new promising approach in biodiversity is the science of metabolomics. Metabolomics is an emergent field in the "omics" research area and is related to global characterization of small metabolic molecules in biological systems. Metabolomics is the last expression of the cellular regulation resulting in the visible phenotypes. A research method for metabolomics is spectroscopy in the near infrared (NIRS). The NIRS technology was developed in the mid-1990s by the United States Department of Agriculture. It was first used to quantify protein levels in wheat and fat/oil levels in soybeans in order to facilitate payment of farmers by grain cooperatives and for rapid segregation of wheat in different silos given that conventional methods were time consuming.

The NIRS is a type of vibrational spectroscopy that uses light energy from wavelengths corresponding to 750 to 2500 nm. The interaction of light with matter in those frequencies can provide qualitative and quantitative information at the molecular level. Advantages of this technique are: universal application (works on any molecule containing the connections C-H, N-H, O-H and S-H); it is a fast method (one minute or less per sample); it doesn't generate solid, liquid or gaseous residues; it is a clean technology (environmentally friendly); it uses small samples, in situ or alive, no previous sample treatment is needed and importantly it is neither invasive nor destructive. NIRS data analysis requires statistical software for sample identification, qualification, and quantification of the entities being studied.

Application of NIR as an analysis tool can be found in practically all areas, from astronomy, industry, quality control, environment, taxonomy, and medicine. NIRS has been used to identify several coleopteran species (Dowell et al., 1999), detect parasitized weevils in wheat kernels (Baker et al., 1999), and to detect external and internal insect infestation in wheat (Ridgway & Chambers, 1996; Ghaedian & Wehling, 1997; Dowell et al., 1998).

In Brazil, the application of NIRS was mainly for soil analysis and quality control of medicines, lubricants, and other products. Nowadays, NIRS is broadly used in industry and in scientific research around the world because it is a fast and reliable technique for measurement, quality-control and for analytic process technology. Lazzari et al. (2009) demonstrated patterns of diversity in Psocoptera using near infrared spectroscopy. The objective of the present work is to demonstrate that metabolomics, by spectroscopy in the NIR, is a fast non-destructive and robust strategy to test and organize hypotheses related to sex and species determination and diversity patterns of Psocoptera.

2. Materials and methods

Samples of ten species of Liposcelis (Psocoptera: Liposcelididae) from laboratory cultures were investigated: *L. bostrychophila* Badonnel, 1931; *L. brunnea* Motschulsky, 1852; *L. corrodens* (Heymons, 1909); *L. decolor* (Pearman, 1925); *L. entomophila* (Enderlein, 1907); *L. fusciceps* Badonnel, 1968; *L. granicola* Broadhead & Hobby, 1944; *L. paeta* Pearman, 1942; *L. pearmani* Lienhard, 1990; and *L. rufa* Broadhead, 1950. The previous identification of the species was accomplished based on the key by Opit et al. (2008) for *Psocoptera* of grains and stored products. This identification key is based on the infrageneric classification proposed by Badonnel where the species are placed in a section, group and subgroup using morphological characters (Mockford, 1993). For the NIRS analyses, the reflectance spectra (R) were obtained in a spectrometer of the series Excalibur Bio-Rad FTS 3500GX (Bio-Rad Laboratories, Cambridge, MA, USA); equipped with KBr beam splitter; detector of deuterate triglicerin sulfate (DTGS); radiation source of silicon carbeto; and accessory of diffuse reflectance in the near infrared ranges from 7500 to 4000 cm-1 (1428 to 2500 nm) with a resolution of 1 cm-1.

Each insect was positioned directly on the accessory of diffuse reflectance and a total of 64 readings were obtained for each insect in each spectrum, using ten replicates for each species. Processing of the spectra used the first Savistky-Golay derivative (21 point window and second order polynomial) and smoothing (seven point window, The Unscrambler™ version 9.1, Camo Software AS, Oslo, Norway). Discriminant analysis (DA) was then applied to the spectra to evaluate the discrimination between groups (sex, species, and sex + species) proposed by NIR spectroscopy (JMP™ version 8.0.1, SAS Institute, Cary, NC, USA).

3. Results

Using discriminant analysis, we tested the NIRS data for sexual dimorphism, species discrimination and the combination of species and sex of psocids. The analysis separates the sexes for all individuals independent of species with 100% resolution. There is high probability that a given individual can be correctly placed in a given group based on its sex (Fig. 1). The next analysis was to discriminate each species. The results showed that there was practically no overlapping of patterns between species when their NIR spectral data were analyzed by discriminant analysis (Fig. 2). Finally, the discriminant analysis showed that the NIR spectral data can give sufficient information to discriminate simultaneously sex and species with 100% resolution (Fig. 3).

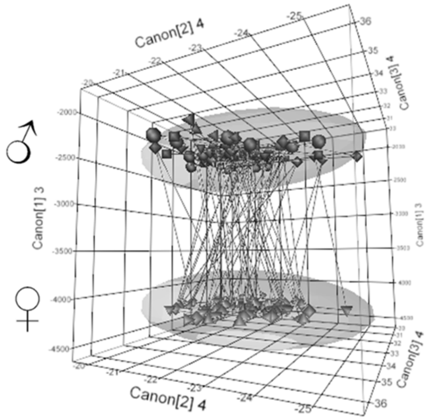

Figure 1 Sexual discrimination based on discriminant analysis of NIRS data for ten species within the genus Liposcelis. Clear spheres represent the males and dark triangles the females. Male and female of each species are connected by lines. FD1, FD2 and FD3 are the discriminant factors 1, 2 and 3, respectively.

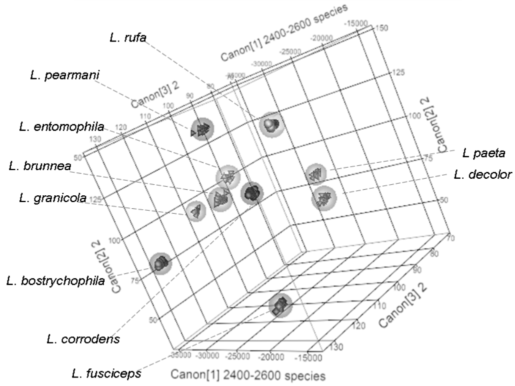

Figure 2 Species discrimination based on discriminant analysis of NIRS data for ten species within the genus Liposcelis. Groups of the same symbols locked by ellipses represent different species. Probabilities of each ellipsis in each species are > 90%. FD1, FD2 and FD3 are the discriminant factors 1, 2 and 3, respectively.

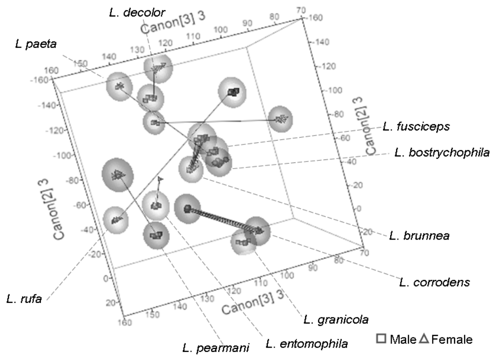

Figure 3 Simultaneous discrimination of sex and species within the genus Liposcelis based on discriminant analysis of NIRS data. Groups of the same symbols locked by ellipses represent different species and sex. Probabilities of each ellipsis for each species are between 82% (larger circular areas - more disperse) and 92% (smaller circles). Male and female of each species are connected by lines. FD1, FD2 and FD3 are the discriminant factors 1, 2 and 3, respectively.

4. Discussion

The order Psocoptera is divided in three suborders: Trogiomorpha, Troctomorpha and Psocomorpha. The suborder Troctomorpha, that contains the family Liposcelididae, consists of two infraorders and eight families. Members of the family Liposcelididae are small, flattened psocids, with enlarged hind femora; when wings are present, venation is reduced; in alate forms both fore and hind wings are present; eyes near vertex; in apterous forms eyes remote from vertex, each consisting of two large elements alone or preceded by six or fewer small ocelloids; pronotum lobed; thoracic sterna broad and bearing cilia. According to the Psocoptera World Catalogue (Lienhard and Smithers, 2002), the species *L. bostrychophila*, *L. fusciceps* and *L. lenkoi* have already been recorded in Brazil along with other 370 Psocoptera species, in 85 genera and 29 families - most of them have been poorly studied.

The integument, based on its composition, is a part of the phenotypic expression of the insect. Thus, it can be considered as a character that is as valuable as other phenotypic or genotypic expressions, such as ethnology, morphology or molecular biology. Entomological literature demonstrates the importance of cuticular hydrocarbons in insects (Lazzari et al., 1991) and other arthropods; these function as a barrier that protects the insect against desiccation, entrance of microorganisms, and other numerous biochemical, physiological, semiochemical, and intraspecific and interspecific ecological functions (Howard and Blomquist, 2005). NIRS does not only detect the cuticular hydrocarbons, but it also analyses other molecules present in small amounts that possess the groups O-H, C-H, N-H and C=O in some part of their structure (Kradjel, 1991).

The great resolution of NIRS to discriminate *Liposcelis* species demonstrated herein by the multivariate analysis is corroborated by studies of other insect taxa (Benedict, 1955; Dowell et al., 1999; Stackebrandt et al., 2002; Cole et al., 2003; Ami et al., 2004; Paliwal et al., 2004; Zhao et al., 2006; Aldrich et al., 2007). Therefore, NIRS can be considered as a viable technique for insect species identification. It is pertinent to mention that the report of the committee for revision of the definition of species in bacteriology (Stackebrandt et al., 2002) includes the use of NIRS techniques for systematic studies of prokaryotes. Considering the literature that reports the application of NIRS in taxonomy and systematics of prokaryotes, we suggest that NIRS has an even greater potential as a novel technique in the systematics of eukaryotes.

The delimitation of species is a topic of constant discussion and refinement (Wiens, 2008). If NIRS has the capacity to recognize the identity of a given species, as shown in this study for the species of Liposcelis, NIRS could also contribute to defining whether the extension of the variability in the spectrum is evidence of the intraspecific variability of a given species, such as sex discrimination. It is already clear that spectrum variability can be considered as evidence of interspecific variation; therefore, NIRS would be a valuable tool for the discovery of cryptic species.

The NIRS technique is a promising alternative for distinguishing between not only genera and species of Psocoptera, but as shown in this study, for discriminating males from females of each species simultaneously (Fig. 3). One of our objectives was simply evaluating sexual variation not other types of intraspecific variation. Although, the intraspecific variation not related to sex may be noticed in the three figures presented by the dispersion of each point (specimen) from the centroid of its group (sex or species). We calibrated the analysis based on regions of the spectrum that would give us the highest possible discrimination between groups (sex, species, and both combined); however, if one wants to investigate ecological or evolutionary relationships among groups, other spectral regions could be analyzed to complement the information on affinities among the groups. Therefore, in this paper, only autapomorphic and synapomorphic characters to discriminate species and sex were evidenced, but much more information can be obtained.

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