ChemSearch Journal 4(1): 51 – 56, June, 2013
Publication of Chemical Society of Nigeria, Kano Chapter

Received: 12/04/2013 Accepted: 16/06/2013

GC – MS Characterization of Degutted White Grubs' Fatty Acids Composition

***A. J. Alhassan and M. S. Sule**
Department of Biochemistry, Bayero University, P. M. B. 3011, Kano
Email: ajalhassan@yahoo.com

ABSTRACT

Fatty acids composition of white grubs examined by GC- MS identified 19 different fatty acids; 11 saturated, 7 monoene and a cyclopropaneoctanoate. The identified ones are Methyl tetradecanoate (C14:0), Methyl dodecanoate (C12:0), Methyl cis – 9 - octadecenote (C18:1), Methyl(7E) – 7 – hexadecenoate (C16:1), Methyl palmitoleinate (C16:1), Methyl 8 –(2-hexylcyclopropyl)octanoate, Methyl (7E)-7 – Octadecenoate (C18:1), Methyl 14 – methylpentadecanoate (C15:0), n–Hexadecanoic acid methyl ester(C16:0), n- octadecanoic acid methyl ester (C18:0), n- pentadecanoic acid methyl ester (C15: 0), Methyl trans – 9- octadecenoate (C18:1), Methyl (10E) – 10 –octadecenoate (C18:1), Methyl heneicosanoate (C21:0), Arachidic acid methyl ester (C20:0), Methyl – 15 – metylhexadecanoate (C16:0), Methyl tridecanoate (C13:0). The presence of eicosanoic acid indicates that white grubs may be rich in essential fatty acids. The result established a hybrid nature in fatty acid composition of the grubs in that it has both plant and animal specific fatty acids. The presence of saturated fatty acids and precursor for some physiologically active lipids may qualify WGs lipid to serve dietary and medicinal role.

Keywords: Fats, Fatty acids, GC – MS, White Grubs

Introduction

Naturally many different fatty acids exist and others are produced as artifacts when fats are used in processing processes (Christie, 1998). The technique of gas chromatography (GC) revolutionized the study of lipids by making it possible to determine the complete fatty acid composition of a lipid in a very short time. Generally fatty acids occur either in free (unesterified) state, or as esters, of glycerol, cholesterol or long-chain aliphatic alcohols, and as amides in sphingolipids. Aliphatic carboxylic acids are often more conveniently examined as their methyl or trimethylsilyl esters because of their volatility and gas chromatographic behaviour are much suitable for their mass spectral studies. For other techniques, notably high-performance liquid chromatography (HPLC), alternative derivatives such as those with UV chromophores, are better (Christie, 1998). Picolinyl (3 - hydroxymethylpyridinyl) esters or pyrrolidide (4, 4-dimethyloxazoline (DMOX)) (Kitson *et al.*, 1996; Christie, 1998) derivatives have special properties for GC-mass spectrometry (MS) of fatty acids.

White grubs (WGs) are larvae of beetles found in waste deposit (Alhassan *et al.,* 2009). Though, white grub is seen and / or presented in the world's field of science as more or less a pest, with less or no positive economic importance, recent research and discoveries show that they can also serve as food (Alhassan *et al.,* 2009), for example among the Ibos of Eastern Nigeria, and as medicine among the Hausa/ Fulani in Northern Nigerian (Personal interview, 2008). White grubs among the Hausa/Fulani are called *Gwazarma* or *Dole-Dole*. It is used in the treatment of jaundice, fever and general body weakness. The Ibos call white grubs as *Eruru*, cheaply obtained from palm trees and moist-refuge dump and it is used as food, while Benin people of Edo State, Nigeria call it *Okhio*. They extract fats from the larvae to process other foods. It can also be eaten directly as food. Some communities dry the flesh part of the grub and grind it to powder for preparing spices and pulp, based on the belief that it has some medicinal effects. A preliminary study has shown that white grubs is rich in protein and fats (Alhassan *et al.,* 2009), characterizing these nutrients could provide information on the nutritional and medicinal properties of the grubs. This research is to characterize the fatty acid content of WGs fats using GC – MS method of analysis.

Material and Methods

Sample Collection and Preparation for Analysis

White grubs (WGs) used for this study were collected from public wastes of Darmanawa quarters, Tarauni Local Government, Kano State,

Nigeria in the months of July and August, 2010. Whole WGs were cleaned of dirt, blot, degutted and oven dried for at least six hours at 105˚C, before being ground to powder and kept in plastic container at 25ºC.

The fat was extracted using acetone by sohxlet methods as described by Harold *et al* (1981). Derivertization and GC – MS analysis (using GC –MS – QP2010 SHIMADZU, JAPAN) followed alkaline hydrolysis of 0.2 g of the extracted fat as described by Rexanka *et al* (1990), methyl ester of the corresponding fatty acids were prepared according to Kitson *et al* (1996) by using 0.3 mg sodium sulphate, 2 cm^3 of n-hexane/dimethylcarbonate mixture (1:1) and 1 cm^3 of sodium methylate and shaken for one minute. To the whole preparation 3 cm^3 water was added with shaking and finally centrifuged at 2500 rpm for 3 minutes, and the filtrate was used for GC – MS analysis by aspirating to the column via an inlet where the heat chamber acts to volatize the sample. Carrier gas acts to transport the sample into capillary column and the molecules in the analytes were separated as they moved along the length of the column due to differences in their chemical properties. The molecules elute separately from the gas chromatograph owing to the differences in their retention time. The eluted molecule is captured, ionized, accelerated, deflected and detected by the mass spectrometer, by breaking each molecule into ionized fragments and the fragments were detected using their mass to charge ratio by the detector.

Result and Discussion

The mass spectrum of methyl ester derivative of 15 – methylhexadecanoic acid is illustrated in Figure 1, the most abundant ions from the methyl esters are $(M - OCH_3)^+$, is the Maclafferty peak at m/z = 74 due to formation of $H_2C = C(OH)OCH_3^+$ and the β – cleavage peak at m/z = 87 due to the formation of $^+CH – CH_2COOCH_3$ ion. The spectra is typical for saturated fatty acids in that it has the prominent ions at m/z = 41, 43, 57, 74 and 87, which are all fragment ions about the methyl group. The molecular ion (m/z = 284) fragments by loss of m/z = 43 to m/z 241. The spectra may be interpreted as; loss of a methylene group to m/z = 227, then a gap of 28 atomic mass unit (amu) to m/z = 199 and a further successive lost of ethylene gives m/z = 185, 171, 157 and 143. The loss of water (H_2O) to m/z = 125. Then a gap of 24 amu to m/z = 101, further loss of methylene to m/z 87 and a gap of 13 amu to m/z = 74, which lost hydroxyl group to m/z = 57 (C_2H_5CO). It then fragments by either loss of methylene to m/z = 43 (CH_3CO) or a gap of 16 amu to m/z = 41 and they both fragments to m/z = 27 ($C_3\dot{H}_5$) by respective gap of 16 amu and loss of methylene.

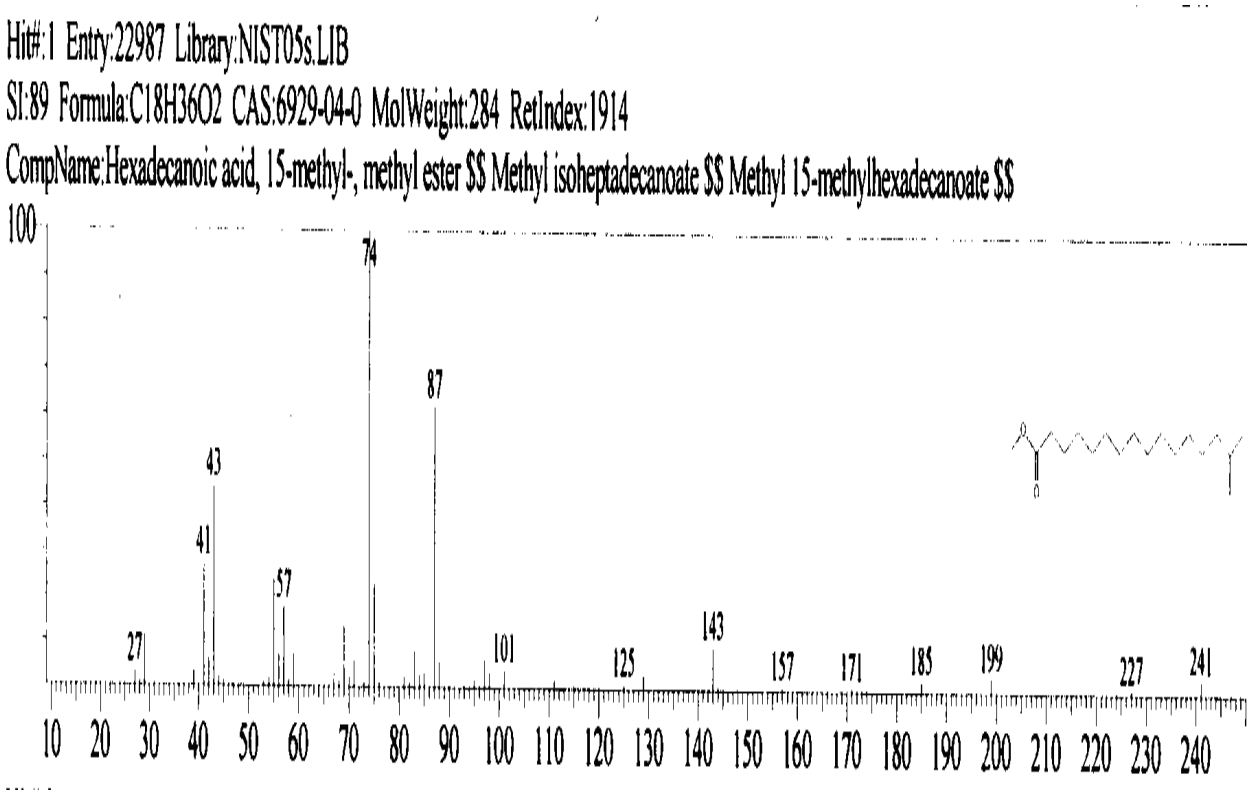

Figure1: The mass spectrum of methyl derivative of 15 – methylhexadecanoate

The EIMS of unsaturated fatty acids shown in Figure 2 are very different from those of their saturated analogues, and they also vary a little according to degree of unsaturation. When double bond is reached, there is a gap 26 amu (Christie, 1998). This gap can sometimes be difficult to locate precisely, but a further distinctive feature of clear diagnostic value is two abundant ions 14 amu apart, representing cleavage at the distal side of the double bond (Harvey, 1992).

The mass spectrum of methyl cis -9-octadecenoic acid is illustrated in Figure 2. The prominent molecular ion on the spectrum (m/z = 264) formed from the parent ion ($[CH_3 (CH_2)_7CH = CH (CH_2)_7CO\text{-}OCH_3^+$) by loss of 32 amu ($CH_3OH$], followed by series of ions 42 amu apart for the cleavage at successive propylene groups. Then a gap of 43 amu to m/z = 137, followed by gaps of 14, 25, 11, 13, 5, amu to fragment ions at m/z = 123, 98, 87, 74 and 69 respectively and series of ions 14 amu apart for cleavage at successive methylene group as m/z = 55, 41 and 27.

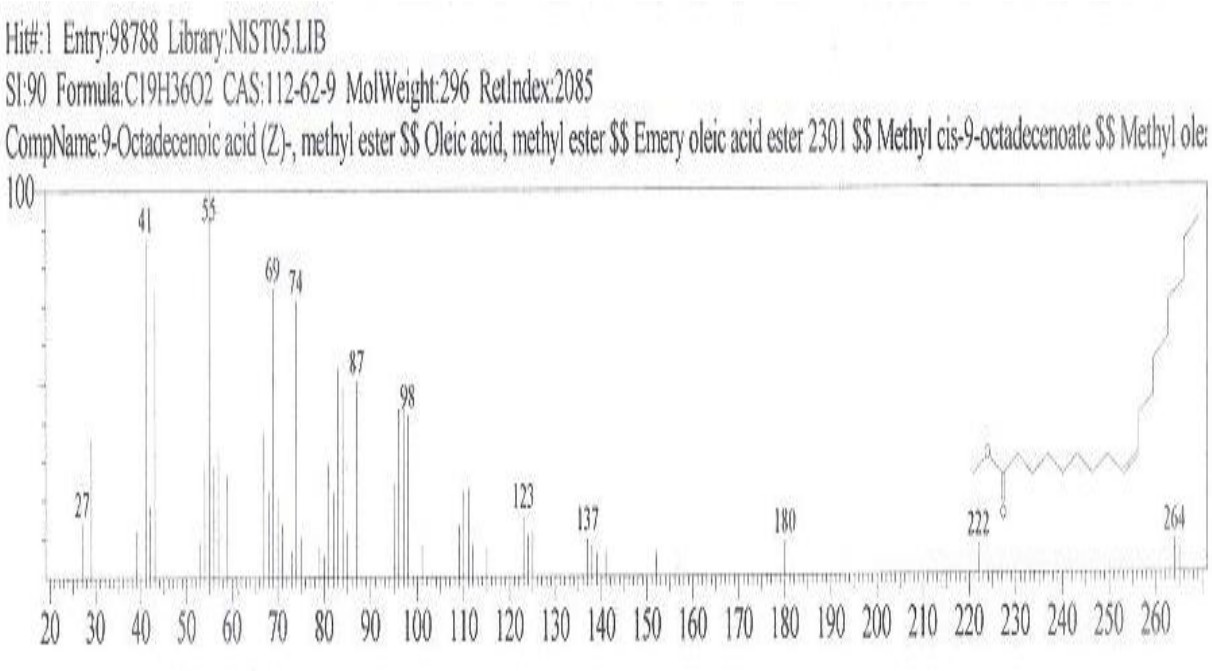

Figure 2: The mass spectrum of methyl derivative of cis-9-octadecenoate

Table 1 presents the varying chain length fatty acid contents of degutted white grubs and the compounds identified by EIMS, their formulae and the corresponding fatty acids (saturated and unsaturated).

Table 1: Fatty Acid Composition of Degutted White Grubs Fats

R.t. (min)	Compounds Identified and their formulae	Corresponding acid
13.12	Methyl 15 – metylhexadecanoate $CH_3OCO(CH_2)_{14}CHCH_3$	Hexadecanoate C16:0
13.12	Methyl tridecanoate $CH_3OCO(CH_2)_{11}CH_3$	Tridecanoate C13:0
13.12	Methyl tetradecanoate $CH_3OCO(CH_2)_{12}CH_3$	Tetradecanoate C14:0
13.12	Methyl dodecanoate $CH_3OCO(CH_2)_{10}CH_3$	Dodecanoate C12:0
16.97	Methyl cis – 9 - octadecenote $CH_3OCO(CH_2)_7CH = CH(CH_2)_7CH_3$	Cis - 9 - octadecenote C18:1
14.99	Methyl(7E) – 7 – hexadecenoate $CH_3OCO(CH_2)_5CH = CH(CH_2)_6CH_3$	7 – hexadecenoate C16:1
14.99	Methyl palmitoleinate $CH_3OCO(CH_2)_7CH = CH(CH_2)_5CH_3$	9 – Hexadecenoate C16:1
14.99	Methyl 8 –(2-hexylcyclopropyl)octanoate	Cyclopropaneoctanoate
14.99	Methyl (7E)-7 – Octadecenoate $CH_3OCO(CH_2)_5CH = CH(CH_2)_8CH_3$	7 – Octadecenoate
15.33	Methyl 14 – methylpentadecanoate $CH_3OCO(CH_2)_{13}CHCH_3$	Pentadecanoate C15:0
15.33	n–Hexadecanoic acid methyl ester $CH_3OCO(CH_2)_{14}CH_3$	Hexadecanoate C16:0
15.33	n- octadecanoic acid methyl ester $CH_3OCO(CH_2)_{16}CH_3$	Octadecanoate C18:0
15.33	n- pentadecanoic acid methyl ester $CH_3OCO(CH_2)_{13}CH_3$	Pentadecanoate C15: 0
16.97	Methyl trans – 9- octadecenoate $CH_3OCO(CH_2)_7CH = CH(CH_2)_7CH_3$	Trans - 9 - octadecenoate C18:1
16.97	Methy (10E) – 10 –octadecenoate $CH_3OCO(CH_2)_8CH = CH(CH_2)_6CH_3$	10 –Octadecenoate C18:1
17.15	Methyl heneicosanoate $CH_3OCO(CH_2)_{19}CH_3$	Heneicosanoate C21:0
17.15	Arachidic acid methyl ester $CH_3OCO(CH_2)_{18}CH_3$	Eicosanoate C20:0

GC – MS analysis of white grubs' lipids indicated the presence of fatty acids that varies in chain length, presence and position of double bond(s), including the saturated fatty acids; 15 – methyl hexadecanoic acid, tridecanoic acid, methyl tetradecanoic acid, methyl dodecanoic acid, 14 – methyl pentadecanoic acid, hexadecanoic acid, octadecanoic acid, pentadecanoic acid, henicosanoate ($CH_3(CH_2)_{19}COOCH_3$), 14 – methyl pentadecanoic acid, eicosanoic acid and the unsaturated fatty acids; cis -9- octadecenoic acid, 7 – hexadecenoic, 9 – hexadecenoic acid, 8– (2 hexyl cyclopropyl) octanoate, 7 - octadecenoic acid, methyl (10E) 10 - octadecenoic acid and methyl - trans -9- octadecenoic acid.

This contradicts the fact that the common fatty acids of animal and plant origin have even numbered chains of 16 -22 carbon atoms, with zero to six double bonds of the *cis* configuration; methylene – interrupted double bond systems predominate. These echo well with statement by Badami and Patil (1981) that countless exceptions exist in nature, odd and even – numbered fatty acids with up to nearly 100 carbon atoms exist and the double bonds can be of the *trans* configuration, acetylenic and allenic bonds occur, and there can be innumerable other structural features, including branch point, rings, oxygenated functions, and many more. This contradiction should be verified by either shade drying or oven drying at 60^{o}C, the

WGs followed by cold extraction of the fat and compared with the result of this finding.

Based on this finding it may be inferred that white grub could play a significant role in medicine and nutrition. The ranges of fatty acids detected ranged from those specifically found in some organisms to those found in both plants and animals and some that are neither of plant nor animal origin, such as tridecanoic acid (C13), 14 – methyl pentadecanoic acid (C15) and pentadecanoic acid (C15). The fatty acid methyl dodecanoic acid (C12) resembles lauric acid (C12) in chain length, except the branched methyl group. Lauric acid is specifically found in spermaceti, cinnamon, palm kernel, coconut oil, laurels and butter, and the fatty acid, methyl tetradecanoic acid (C14), resembles myristic acid (C14) specifically found in nutmeg, palm kernel, coconut oils, myrtles and butter (Mayes and Botham, 2003). The fatty acids; palmitate (C16) and Stearic (C18) acid, which are common in all animal and plant (Mayes and Botham, 2003), were also detected in white grubs i.e. hexadecanoic (C16), octadecanoic (C18) acids. White grubs fat shows peculiar nature in containing fatty acids that are either plant or animal specific.

The unsaturated fatty acids detected in white grubs are monoenoic acids (one double bond); *cis*-9-hexadecenoic **(**16:1;9 ω ; palmitoleic) found in nearly all fats, *cis*-9-octadecenoic (18:1;9 ω; oleic), possibly the most common fatty acid in natural fats and *trans*-9-octadecenoic (18:1;9 ω; elaidic) mostly found in hydrogenated and ruminant fats (Mayes and Botham, 2003). Other monoenoic acids found in white grubs that are not commonly found in natural fat are the 7 – hexadecenoic, 8– (2 hexyl cyclopropyl) octanoate, methyl (10E) 10 - octadecenoic acid and 7 - octadecenoic acid that may have certain physiological roles. These fatty acids play significant roles in living system ranging from energy generation and reserved, and as components of structural and specialized lipids.

Although, none of the essential fatty acids were detected by the study, however eicosanoic acid (arachidic acid**)** was detected**.** eicosanoic acid is known to be derived from the essential fatty acids, linoleate and linolenate**,** or directly from dietary arachidonate and eicosapentaenoate. Arachidonate is usually derived from the 2 position of phospholipids in the plasma membrane by the action of phospholipase A2 and also from the diet. These may indicate the possible presence of some of these essential fatty acids in white grubs, which could have been detected if any one of these; pyrrolidides, picolinyl (3 – hydroxymethylpyridinyl) ester and 4, 4-dimethyloxazoline (DMOX) were used as derivatizing agents (Christie, 1998).

Arachidonate and some other C20 polyunsaturated fatty acids give rise to three groups of eicosanoids, known as prostaglandins (PG), thromboxanes (TX) and leukotrienes (LT), and lipoxins (LX) which are physiologically and pharmacologically active compounds (Bhagavan, 2002). Physiologically, they are considered to act as local hormones functioning through G-protein-linked receptors to elicit their biochemical effects. Thromboxanes cause vasoconstriction and platelet aggregation. Prostacyclins (PGI2) are produced by blood vessel walls and are potent inhibitors of platelet aggregation. Thus, thromboxanes and prostacyclins are antagonistic. PG3 and TX3, formed from eicosapentaenoic acid (EPA) in fish oils, inhibit the release of arachidonate from phospholipids and the formation of PG2 and TX2. PGI3 is as potent an antiaggregator of platelets as PGI2, but TXA3 is a weaker aggregator than TXA2, changing the balance of activity and favoring longer clotting times. As little as 1 ng/cm^3 of plasma prostaglandins causes contraction of smooth muscle in animals. Potential therapeutic uses include prevention of conception, induction of labor at term, termination of pregnancy, prevention or alleviation of gastric ulcers, control of inflammation and of blood pressure, and relief of asthma and nasal congestion. In addition, PGD2 is a potent sleep-promoting substance. Prostaglandins increase cAMP in platelets, thyroid, corpus luteum, fetal bone, adenohypophysis, and lung but reduce cAMP in renal tubule cells and adipose tissue. The above pharmacological and physiological effects could be associated with ingestion of white grub fats which may contain some of these substances.

White grubs fat was shown to contain a precursor to leukotrienes and lipoxins, it may therefore regulate many disease processes. The low-reacting substance of anaphylaxis (SRS-A) being a mixture of leukotrienes C4, D4, and E4. This mixture of leukotrienes is a potent constrictor of the bronchial airway musculature. These leukotrienes together with leukotriene B4 also cause vascular permeability and attraction and activation of leukocytes and are important regulators in many diseases involving inflammatory or immediate hypersensitivity reactions, such as asthma. Leukotrienes are vasoactive, and 5-lipoxygenase has been found in arterial walls. Evidence supports a role for lipoxins in vasoactive and immunoregulatory function, as counterregulatory compounds (chalones) of the immune response. White grubs fats may served both nutritional and medicinal purposes.

Reference

Alhassan, A. J., Sule, M. S., Hassan, J. A., Baba, B. A. and Aliyu, M. D. (2009). Proximate and Elemental Composition of White Grubs. *Bayero Journal of Pure and Applied Sciences*, 2(2): 188 – 190.

Badami, R.C. and Patil, K. B., (1981) Prog. *Lipid Res.*, 19, 119-153.

Bhagavan, N. V. (2002). Medical Biochemistry 4th Ed. Harcourt/Academic Press, San Diego California Pp 387 – 400.

Christie, W.W. (1989) HPLC and GC–Mass Spectrometry in the Analysis of Fatty Acids. In: *Fats for the Future* (Cambie, R.C., ed.) Ellis Horwood, Chichester. Pp. 335–344.

Christie, W. W., (1998) Review: Gas Chromatography–Mass Spectrometry Methods for Structural Analysis of Fatty Acids. *Lipids*, **3:**343 -353.

Harold E. Kirk, R. S ans Sawyer, R. (1981) Nitrogen and Crude Protein. In: *Pearson's Chemical Analysis of Food* 8th ed. Churchill Livingstone. Pp 15 – 23.

Harvey, D.J. (1992) Mass Spectrometry of Picolinyl and Other Nitrogen Containing Derivatives of Fatty Acids, in *Advances in Lipid Methodology—One* (Christie, W.W., ed.), Oily Press, Dundee, pp.19–80.

Kitson, F. G., Larsen, B. S. and McEwen, C. N. (1996) Gas Chromatography and Mass Spectrometry a Practical Guide. Academic Press, San Diego California. Pp 3 – 237.

Mayes, P. A. and Botham, K. M., (2003) Lipids of Physiologic Significance In: *Harper's Illustrated Biochemistry*. Murray, R. K., Granner, D. K.. Mayes, P. A., and Rodwell, V. W. 26 ed.,) Lange Medical Books/McGraw-Hill, Medical Publishing Division Pp 111 – 120.

Rexanka, T., Sokolov, M. Yu. and Viden, I. (1990) Fat Electron Mass Spectroscopy (FEMS) Microbial. *Ecol.* 73, 231 .