

The foveola of *Bothriochloa alta* (Poaceae: Andropogoneae): Extrafloral nectary or secretory gland of essential oils?

Lidia R. Scrivanti^{a,c}, Gabriel Bernardello^{a,b}, Ana M. Anton^{a,b,*}

^aInstituto Multidisciplinario de Biología Vegetal (CONICET – Universidad Nacional de Córdoba), Casilla de Correo 495, 5000 Córdoba, Argentina

^bMiembro de la Carrera del Investigador, CONICET, Argentina

^cSECyT-UNC, Argentina

Received 22 June 2006; accepted 11 December 2006

Abstract

In some *Bothriochloa* species, the foveola or pit – a depression located in the lower glume of the sessile spikelet – has been interpreted as being an extrafloral nectary, although neither the structure has been studied nor the secretion was chemically characterized. On this basis, we analyzed the characteristics and structure of the foveola and the chemical composition of the secretion in *Bothriochloa alta* (Hitcch.) Herter, a grass with disjunct distribution from North and South America. In parallel, inflorescence visitors were identified during three summers (2003–2005) in populations from the Province of Córdoba (Argentina). The results show that the foveola functions as a secretory cavity producing essential oils instead of being an extrafloral nectary as previously suggested. The main compounds identified as components of the oils included methyl linoleate ester (34%), and two oxygenated sesquiterpenes: tau-cadinol (23.3%) and 6-methyl- α (E)-ionone (9.8%). Several species of Coleoptera, Hemiptera, and Diptera are inflorescence visitors.

© 2008 Elsevier GmbH. All rights reserved.

Keywords: Essential oil; Secretory gland; Sesquiterpenes; Visitors

Introduction

Nectaries are special structures devoted to nectar secretion, being diverse in shape, structure and function (Fahn, 1979; Elias, 1983; Pacini et al., 2003). According to their position, two main types are recognized: floral and extrafloral nectaries (Fahn, 1979; Elias, 1983;

Smets, 1986). Generally, floral nectaries are involved in pollination whereas the extrafloral ones might be devoted to the protection of vegetative and reproductive structures from herbivory. Floral nectaries recognized in Monocots are mainly gynopleural (septal) and perigonal (Smets et al., 2000), and they have not been registered in Poaceae so far. However, different types of extrafloral nectaries have been recorded: few species of *Panicum* section *Clavelligera* (Kabuye and Word, 1969) and *Andropogon gayanus* var. *bisquamulatus* (Bowden, 1971) have glandular trichomes – the first on the leaves and pedicels, the second on the leaves – while crateriform glands have been described on the nerves of

*Corresponding author at: Instituto Multidisciplinario de Biología Vegetal (CONICET-Universidad Nacional de Córdoba), Casilla de Correo 495, 5000 Córdoba, Argentina.

E-mail addresses: rscrivanti@imbiv.unc.edu.ar (L.R. Scrivanti), anton@imbiv.unc.edu.ar (A.M. Anton).

sheaths, blades, glumes, lemmas, peduncles, inflorescence branches, pedicels or behind the nodes in *Eragrostis* spp. (Nicora, 1941; Negritto et al., 2003), *Tridens* (Anton, 1977), *Bambusa* (Blüthgen and Reifentath, 2003) and *Panicum* section *Stolonifera* (Zuloaga and Sendulsky, 1988; Morrone and Zuloaga, 1991).

Some *Bothriochloa* species have been supposed to have extrafloral nectaries. This genus of 35–40 species mostly of tropical and subtropical regions (Watson and Dallwitz, 1992) has nearly 28 native species in the Americas (Vega, 2000). As a typical Andropogoneae, it has paired spikelets, the rachis joint and the pedicellate spikelet falling together with the awned, sessile spikelet. The panicles, up to 30 cm long, remain frequently enclosed (kinked) for compression in the sheath of the flag leaf (Gould, 1975). The sessile spikelets are 2-flowered (functionally 1-flowered), fertile and invested with a hairy callus, the pedicellate being either male or sterile. In some species, the lower (inferior) glume exhibits a central pit or depression (foveola) that has been interpreted as an extrafloral nectary (Van der Pijl, 1982), even though the exudate in nature has never been recorded (Vega, 2000). This is the case of *Bothriochloa alta* (Hitchc.) Henrard (“tall bluestem”), a disjunct perennial from North (Western Texas and Southern New Mexico to West Central Mexico) and South America (Bolivia and Argentina), found in sandy and rocky soils between 400 and 2900 m a.s.l.

This paper aims to analyze the morphology and anatomy of the pit, to reveal the chemical nature of the detected secretion and to identify the insect inflorescence visitors in populations from Central Argentina.

Material and methods

Plant material

The *B. alta* plants were collected at flowering stage in the Province of Córdoba, Argentina, during January to April from 2003 to 2005. Vouchers are on deposit at the Herbarium of the Museo Botánico de Córdoba (CORD). The harvesting sites are: *Dept. Capital*: Córdoba, surroundings of Chateau Carreras, *Scrivanti 117* (22-I-2004). *Dept. Colón*: Way to La Calera, *Scrivanti 39* (29-I-2003). *Dept. Punilla*: Villa Carlos Paz, *Scrivanti 198* (08-II-2005), *201* (08-II-2005), *207* (15-II-2005). *Dept. Santa María*: Anisacate, Villa Satyta, *Scrivanti 58* (15-IV-2003), *60* (15-IV-2003), *99* (07-I-2004), *173* (26-IV-2004) y *174* (26-IV-2004). Plants were also cultivated in the experimental garden of the Instituto Multidisciplinario de Biología Vegetal at Córdoba, from the populations *Scrivanti 39*, *99*, *117*, *173*, *198* and *201*.

Histological analysis

The spikelets were fixed in FAA, dehydrated through an ethyl alcohol/xylene series, and embedded in Paraplast. Sections were cut at 10 µm thickness, mounted serially and stained with safranin and astral blue (Maácz and Vagás, 1961).

Chemical analysis

Two techniques for study of the secretion were applied: to detect sugars, and essential oils. The secretion was extracted at random (from 10 spikelets per panicle of a total of three plants per population) and was placed on Whatman no. 1 chromatography paper. Sugar separation was accomplished by gas chromatography (GC) according to Bernardello et al. (1994). The oil obtained was dried over anhydrous sodium sulphate and stored at 0 °C until analysis by GC coupled with mass spectrometry (MS) according to Juliani et al. (2004). The oil components were identified by comparison of their retention indexes, mass spectra with those of authentic samples, by peak enrichment, with published data (Adams, 1995) stored in the mass spectra library of National Institute of Standards and Technology (NIST 3.0) and our mass spectra library which contains references of mass spectra and of retention indices of volatile compounds.

Identification of visitors

The insects visiting the inflorescences were collected between 9 a.m. and 5 p.m. during three flowering periods from 2003 to 2005 and identified by Claudio Sosa and Graciela Valladares (Centro de Investigaciones Entomológicas, UNC).

Results

The pit is located in the upper portion (1.1–1.8 mm from the apex) of the lower glume in the sessile fertile spikelet of *B. alta*. It is oval in outline, with a diameter of 0.1–0.3 mm in (Fig. 1). The uniserial secretory tissue is covered by a thin cuticle and is water supplied by a vascular strand. In a paradermal section, the secretory cells are isodiametric, with straight, smooth and thickened external walls, and dense cytoplasm; a prominent nucleus is displaced to the anticlinal wall (Fig. 2A–C).

Secretion starts between 9 a.m. and 4 p.m. simultaneously in all the spikelets along the panicles of every plant. It is observed as a small drop ca. 1.5–2 µL, filling the whole cavity of the foveola and remaining there for the spikelet lifetime. During this period of time the drop

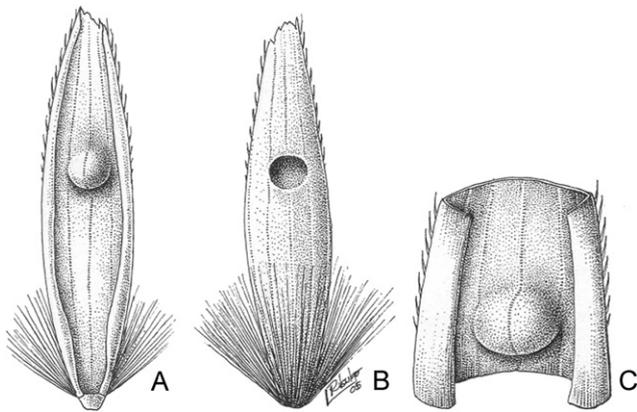


Fig. 1. *Bothriochloa alta* (A, B) lower glume, abaxial and adaxial view. (C) Detail of (A), showing the protuberant pit. Bars = 1.5 mm in (A, B); 0.75 mm in (C).

displays no chemical variation. Chemical analysis (GC/MS) of the secretion reveals the presence of essential oils but no sugars. It is composed of methyl linoleate ester (34%) and the oxygenated sesquiterpenes tau-cadinol (23.3%) and 6-methyl- α (E)-ionone (9.8%). In addition, sesquiterpene residuals as hydrocarbons (19.9%), oxygenated sesquiterpenes (9.5%), monoterpenes (approx. 2.3%), esters (approx. 0.7%), hydrocarbons (0.2%), ketones (approx. 0.1%) and one alcohol (0.1%) has been detected (Table 1).

Different orders of insects have been registered visiting the inflorescences of *B. alta*: Coleoptera, Hemiptera, and Diptera (Table 2). Most of the inflorescences registered were visited by hemipterans followed by coleopterans, which use the panicles to breed and to lay their eggs on the ovaries. In their behaviour, insects do not get in contact with the reproductive organs; in the bodies of the insects examined, there was not any sign of pollen.

Discussion

The foveola of the glume of *B. alta* cannot be considered an extrafloral nectary since its secretion contains no sugars. It is interesting to note that in some grasses in which extrafloral nectaries' secretion has been analyzed (Nicora, 1941; Bowden, 1971; Morrone and Zuloaga, 1991), the characteristic sugars secreted by nectaries, i.e. sucrose, glucose and fructose (Percival, 1961; Baker and Baker, 1983), have been detected.

In general, essential oils are both complex and variable in their chemical composition, being in principle terpenes and their derivatives (Fahn, 1979; Simpson and Ogorzaly, 1995). They have been largely recorded in the grasses, especially in Andropogoneae, *Cymbopogon* being the most representative genus

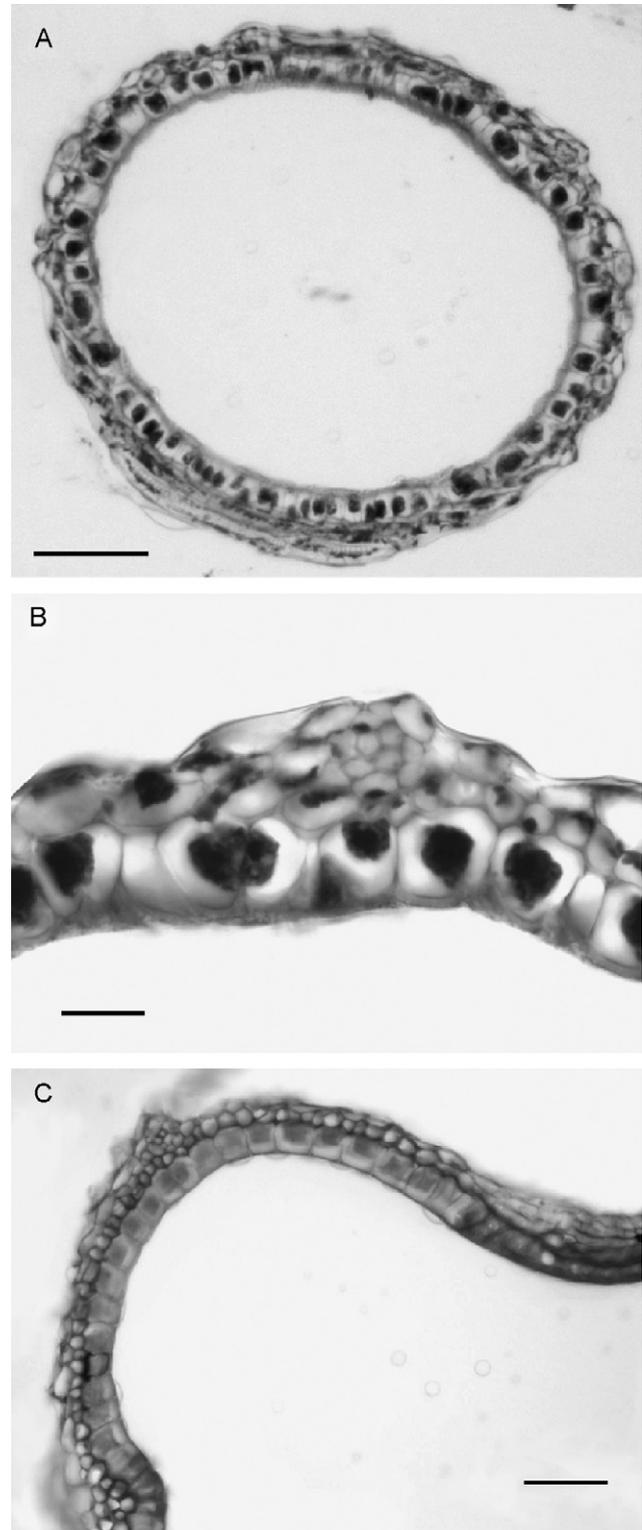


Fig. 2. Paradermal (A) and longitudinal (C) sections of *Bothriochloa alta* foveola showing the secretory tissue. (B) A magnification of (A). Bars = 564 μ m in (A); 141 μ m in (B); 457 μ m in (C).

according to the number of species involved and its commercial importance. The second genus is *Bothriochloa*, with five species studied (Pinder and Kerr,

Table 1. Chemical composition of the exuded essential oil in the foveola in *Bothriochloa alta* (Hitc.) Henrard

Components	Percentages	Retention index
1-Octene	0.1	166
Methyl pentanoate	0.2	190
Tricyclene	Tr	301
Alpha pinene	Tr	319
Octen-3-ol	0.1	383
Beta pinene	0.1	386
3-Octanone	Tr	397
Alpha terpineol	0.1	852
2-Hexenyl butyrate (E)	0.1	864
Piperitone	2.1	1011
Methyl citronellate	Tr	1035
Azulene	Tr	1140
Isobornyl propanoate	0.4	1347
Beta bourbonene	1.2	1355
3-Dodecanone	0.1	1360
Beta cubebene	2.3	1371
1-Tetradecene	0.1	1377
Longifolene	5.5	1404
Beta humulene	1.3	1494
Alpha humulene	1.2	1527
Germacrene D	0.1	1550
Elemol	1.5	1759
Beta cadinene	0.6	1577
Alpha muurolene	1.4	1586
Hydrocarbon sesquiterpene	0.5	1591
Hydrocarbon sesquiterpene	0.5	1598
Valencene	0.1	1624
Cubebol	1.4	1679
6-Methyl-alpha (E)-ionone	9.8	1688
Isobornyl-2-methyl butyrate	Tr	1692
Hydrocarbon sesquiterpene	0.3	1698
Beta vetivenene	1.1	1705
Alpha cadinene	0.6	1733
Alpha calacorene	2.1	1744
Beta calacorene	1.1	1792
Germacrene D-4ol	1.3	1821
Spathulenol	1.1	1825
Gamma eudesmol	0.3	1951
Tau-cadinol	23.3	1973
Alpha muurolol	1.1	1984
Alpha cadinol	2.5	2003
Methyl myristate	Tr	2167
Beta bisabolen-12-ol	0.3	2242
Methyl linoleate	34.0	2915

1980; Kaul and Vats, 1998), but in these cases the material analyzed consisted of the whole plant in flower or only the inflorescences. Interestingly enough, the five *Bothriochloa* species in which essential oils were recorded have no foveola in the glumes.

In general, the insects registered visiting the inflorescences of *B. alta* are frequent floral visitors. However, in the case of *B. alta*, where the anthers are not exposed, their activity cannot be related to pollination. Predation may not be disregarded, since some of the visitors (*Piezodorus guildinii*, for instance) are reputed to be pests in cultivated fields of soybean, alfalfa and maize (Liljestrom and Coviela, 1999; Aragón and Vázquez, 2002). Nevertheless, being this a preliminary report for these aspects, additional work has to be done to determine the behaviour of visitors in this and other foveolate species of *Bothriochloa*.

As a rule, terpenes and their derivatives are produced and secreted by specialized cells (glands) located anywhere in the plant, as in stems, leaves, flowers, etc. (Fahn, 1979). Although their presence might be associated with some special function in the plant, the precise physiological roles have not been defined. However, allelopathic properties and generalized defense functions have been proposed, such as protection against injuries or damages, and herbivore, or to prevent infections from pathogenic agents like bacteria and fungi (Lewinsohn et al., 1991; Phillips and Croteau, 1999; Scrivanti et al., 2003 and references cited therein). Furthermore, other functional and ecological properties of the essential oils have been related to pollinators attraction (Grison-Pigé et al., 1999, 2002).

Grasses as a whole are considered an anemophilous family. However, since pollen is a source of food for many insects, grasses are commonly visited by insects, which suggests the idea of possible insect pollination (Salgado-Labouriau et al., 1993). This occurs mainly in Bambusoid grasses, especially those of the rain forest understory where there is no wind to effect pollination (Soderstrom and Calderón, 1971). However, the location of the foveola in the glume of the fertile spikelet cannot be associated with the attraction of potential pollinators, since *B. alta* is cleistogamous and the stamens are never exposed (the three anthers remain on the top of the mature caryopsis). On the other hand, the secretion of essential oils could provide some kind of

Table 2. Inflorescence visitors in *Bothriochloa alta*

Order	Family	Subfamily	Species
Hemiptera	Pentatomidae	Pentatominae	<i>Piezodorus guildinii</i>
Hemiptera	Pentatomidae	Pentatominae	<i>Dichelops</i> sp.
Coleoptera	Curculionidae	Calendrinae (5 spp.)	
Diptera	Sciaridae (2 spp.)		

protection against insect herbivory during the flowering or fruiting period. Eventually, it could prevent injury or function as an allelopathic substance useful for competition when the diaspore (i.e. the seed plus glumes and glumeles) falls to the ground or during the germination of the seed.

Acknowledgements

We are grateful to Claudio Sosa and Graciela Valladares for insect identification and to Adriana Pérez for technical assistance. CONICET and SECyT-UNC are acknowledged for financial support.

References

- Adams, R.P., 1995. Identification of Essential Oil Components by Gas Chromatography and Mass Spectroscopy. Allured Publishing Corporation Carol Stream, IL.
- Anton, A.M., 1977. Notas críticas sobre Gramíneas de Argentina II. Kurtziana 10, 51–67.
- Aragón, J., Vázquez, J., 2002. Infestación de chinches de maíz. Información para Extensión 74. INTA, Marcos Juárez (Argentina).
- Baker, H.G., Baker, I., 1983. Floral nectar constituents in relation to pollinator type. In: Jones, C.E., Little, R.J. (Eds.), Handbook of Experimental Pollination Biology. Van Nostrand Reinhold, New York, pp. 117–141.
- Bernardello, L., Galetto, L., Rodríguez, I., 1994. Reproductive biology, variability of nectar features and pollination of *Combretum fruticosum* (Combretaceae) in Argentina. Bot. J. Linn. Soc. 114, 293–308.
- Blüthgen, N., Reifenrath, K., 2003. Extrafloral nectaries in an Australian rainforest: structure and distribution. Aust. J. Bot. 51, 515–527.
- Bowden, B., 1971. Studies on *Andropogon gayanus* Kunth. VI. The leaf nectaries of *Andropogon gayanus* var. *bisquamulatus* (Hochst.) Hack. (Gramineae). Bot. J. Linn. Soc. 64, 77–80.
- Elias, T.S., 1983. Extrafloral nectaries: their structure and distribution. In: Bentley, B., Elias, T.E. (Eds.), The Biology of Nectaries. Columbia University Press, New York, pp. 174–203.
- Fahn, A., 1979. Secretory Tissues in Pteridophytes. Academic Press, London.
- Gould, F.W., 1975. The Grasses of Texas. Texas A & M University Press.
- Grisson-Pigé, L., Alice, A., Edwards, A.A., Hossaert-McKey, M., 1999. Interspecies variation in floral fragrances emitted by tropical *Ficus* species. Phytochemistry 52, 1293–1299.
- Grisson-Pigé, L., Hossaert-McKey, M., Greeff, J.M., Bessière, J., 2002. Fig volatile compounds – a first comparative study. Phytochemistry 61, 61–71.
- Juliani, H.R., Zygadlo, J.A., Scrivanti, L.R., De la Sota, E., Simon, J.E., 2004. The essential oil of *Anemia tomentosa* (Savigny) Sw. var. *anthriscifolia* (Schrad.) Mickel. Flavour Fragrance J. 19, 541–543.
- Kabuye, C.H.S., Word, D., 1969. A first record of multicellular gland hairs in the Gramineae. Bot. J. Linn. Soc. 62, 69–70.
- Kaul, V.K., Vats, S.K., 1998. Essential oil composition of *Bothriochloa pertusa* and phyletic relationship in aromatic grasses. Biochem. Syst. Ecol. 26, 347–356.
- Lewinsohn, E., Gijzen, M., Savage, T.J., Croteau, R., 1991. Defense mechanisms of conifers: relationship of monoterpene cyclase activity to anatomical specialization and oleoresin monoterpene content. Plant Physiol. 96, 38–43.
- Liljestrom, G., Coviela, C., 1999. Aspectos de la dinámica poblacional de las chinches *Nezara viridula* y *Piezodorus guildinii* e implicancias en relación a su manejo en el cultivo de la soja. Rev. Soc. Entomol. Argent. 58, 141–149.
- Maáčz, G.J., Vagás, E., 1961. A new method for staining of cellulose and lignified cell walls. Mikroskopie 16, 40–43.
- Morrone, O., Zuloaga, F., 1991. Estudios morfológicos en el subgénero *Dichantherium* de *Panicum* (Poaceae: Paniceae), con especial referencia a *Panicum sabulorum*. Ann. Missouri Bot. Gard. 78, 915–927.
- Negritto, M.A., Durán, M.L., Scrivanti, L.R., Anton, A.M., 2003. Eragrostideae subtribu Eleusininae. In: Anton, A.M., Zuloaga, F.O. (Eds.), Flora Fanerogámica Argentina 86. Córdoba, Argentina, Proflora, pp. 13–68.
- Nicora, E.G., 1941. Glándulas epidérmicas de algunas especies de *Eragrostis*. Darwiniana 5, 316–321.
- Pacini, E., Nepi, M., Vesprini, J.L., 2003. Nectar biodiversity: a short review. Plant Syst. Evol. 238, 7–21.
- Percival, M.S., 1961. Types of nectar in Angiosperms. New Phytol. 60, 235–281.
- Phillips, M.A., Croteau, R., 1999. Resin-based defenses in conifers. Trends Plant Sci. 4, 184–190.
- Pinder, A.R., Kerr, S.K., 1980. The volatile essential oils of five *Bothriochloa* species. Phytochemistry 19, 1871–1873.
- Salgado-Labouriau, M.L., Nilsson, S., Rinaldi, M., 1993. Exine sculpture in *Pariana* pollen (Gramineae). Grana 32, 243–249.
- Scrivanti, L.R., Zunino, P., Zygadlo, J.A., 2003. *Tagetes minuta* and *Schinus molle* essential oils as oxidants agents. Biochem. Syst. Ecol. 31, 563–572.
- Smets, E.F., 1986. Localization and systematic importance of the floral nectaries in the Magnoliata (Dicotyledons). Bull. Jard. Bot. Belg. 56, 51–76.
- Smets, E.F., Ronse Decraene, L.P., Caris, P., Rudall, P.J., 2000. Floral nectarines in monocotyledons: distribution and evolution. In: Wilson, K.L., Morrison, D.A. (Eds.), Monocots, Systematics and Evolution. CSIRO, Melbourne, pp. 230–240.
- Simpson, B.B., Ogorzaly, M.C., 1995. Economic Botany. Plants in Our World. McGraw-Hill, New York.
- Soderstrom, T.R., Calderon, C.E., 1971. Insect pollination in tropical rain forest grasses. Biotropica 3, 1–16.
- Van der Pijl, L., 1982. Principles of Dispersal in Higher Plants, third ed. Springer, Berlin, Heidelberg, pp. 166–168.
- Vega, A.S., 2000. Revisión taxonómica de las especies americanas del género *Bothriochloa* (Poaceae: Panicoideae: Andropogoneae). Darwiniana 38, 127–186.
- Watson, L., Dallwitz, M.J., 1992. The Grass Genera of the World. CAB International.
- Zuloaga, F.O., Sendulsky, T., 1988. A revision of *Panicum* subgenus *Phanopyrum* section *Stolonifera* (Poaceae: Paniceae). Ann. Missouri Bot. Gard. 75, 429–455.