Nonpolar Lipid Composition of *Chenopodium album* Grown in Continuously Cultivated and Nondisturbed Soils

S.R. Leicach[a,b,*], M.A. Yaber Grass[a,b], G.B. Corbino[a,b,c], A.B. Pomilio[b], and A.A. Vitale[b]

[a]Departamento de Biología Aplicada y Alimentos, Facultad de Agronomía, Universidad de Buenos Aires (UBA), Buenos Aires, Argentina, [b]Programa de Plantas Tóxicas y Medicinales–Consejo de Investigaciones Científicas y Técnicas (CONICET), Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, UBA, Ciudad Universitaria, C1428EHA, Buenos Aires, Argentina, and [c]Instituto Nacional de Tecnología Agropecuaria, San Pedro, San Pedro, Buenos Aires, Argentina

ABSTRACT: *Chenopodium album* L. plants grown in continuously cultivated and in nondisturbed soils were compared in terms of the compositions of nonpolar extracts of the corresponding aerial parts. Both light petroleum ether extracts of *C. album* L. were analyzed by high-performance thin-layer LC, capillary GC, and capillary GC-EI-MS. Further percolation and medium-pressure LC, along with EI-MS analysis, permitted the separation and identification of the chemical constituents. Differences were observed between mean contents of the chemical constituents of *C. album* L., with respect to nonpolar extracts, obtained from continuously cultivated and from nondisturbed soils, in particular in linear and branched long-chain hydrocarbons, FA and their esters, and long-chain linear alcohols and aldehydes. The most remarkable features of the disturbed soils were a pronounced increase in the amounts of linear hydrocarbons and a decrease in the relative proportions of FA.

Paper no. L9122 in *Lipids 38,* 567–572 (May 2003).

Owing to the growing world population, crop management involves continuous and intensive exploitation of land based on the use of fertilizers and pesticides in order to achieve higher productivities. The overuse of agrochemicals results in severe alterations to the agroecosystem (1,2), particularly in the physical, chemical, and biological properties of the soil and in groundwater contamination (3–7).

The quality of a soil is defined in terms of its capacity to sustain productivity in natural or managed ecosystems, to maintain or enhance water and air quality, and to support human needs (8). The potential use of some soil properties (organic C; total N; P, K, B, Ca, and Zn concentrations; and cation exchange capacity) and of crop characteristics (total dry matter at physiological maturity, grain yield, kernel number, and prolificacy) as indicators for soil quality, has been demonstrated by multivariate techniques (9). The Rolling Pampa of Argentina (10) has been subjected to continuous cropping in the last two decades, with a nearly total loss of the traditional mixed farming with cattle grazing (11). Thus, soil quality has been reduced by nutrient exploitation, soil compaction, and negative changes in biotic conditions (9,12–16). Different levels of soil deterioration were attributed to farming. Organic C, total N, extractable P, pH, soil aggregate stability (SA_s) and infiltration rate showed the greatest changes compared to the same parameters in pristine conditions (12). Among them, SA_s and P concentration seem to be the most critical parameters in detecting soil deterioration in the Rolling Pampa (9).

Weeds, a group of plants with a great adaptability to environmental changes, have colonized most of the places disturbed by humans, particularly lands undergoing continuous agricultural exploitation. The occurrence of biologically active principles is known in several weed species (17–19); a number are secondary metabolites that are related to the ecological fitness of the producing organism. *Chenopodium album*, a species native to Europe and Asia and called "fat hen" in Europe and "lamb's-quarters" in the United States, causes significant economic loss in agriculture (20). This broadleaf weed grows in association with most common crops in Argentina and has developed cross- and multiple-resistance to synthetic agrochemicals (21). Although *C. album* competes strongly with crops, it also has growth-inhibiting allelopathic effects (22–24), feeding deterrence potential (20), and the ability to control viruses (25), fungi (26), and soil nematodes (27). The bioactive role of *Chenopodium* spp. is appreciated mainly in developing countries as reflected in the use of their raw extracts (18–20). The potential of *Chenopodium* spp. to control pests has been related to a variety of chemicals, such as hydrocarbons (23), saponins (28), flavonoids (29), terpenoids, and steroids (20). Among them, less polar compounds such as hydrocarbons, FA and their methyl esters (30–32), long-chain aldehydes, and alcohols (33) are present in the epicuticular wax layer. Wax deposition plays an important role in reducing water loss; the other major function is related to plant protection against herbivores and pathogens (33). The purpose of our experiment was to compare the nonpolar lipid compositions of *C. album* collected from continuously cultivated and noncultivated areas.

EXPERIMENTAL PROCEDURES

Organic solvents were obtained from Sintorgan (Buenos Aires, Argentina) and were glass-distilled before use; *n*-hexane was freed from olefins followed by distillation. All solvent mixtures are expressed in volumes (vol/vol). Authentic samples were purchased from Sigma-Aldrich.

*To whom correspondence should be addressed at Departamento de Biología Aplicada y Alimentos, Facultad de Agronomía, UBA, Avenida San Martín 4453, C1417DSE, Buenos Aires, Argentina.
E-mail: leicach@mail.agro.uba.ar

Abbreviations: HPTLC, high-performance TLC; MPLC, medium-pressure LC; SA_s, soil aggregate stability; UBA, Universidad de Buenos Aires.

Copyright © 2003 by AOCS Press

Plant sample collection. *Chenopodium album* L. specimens were collected in their vegetative stage during 1998 summertime from a set of fields in the maize production area of Argentina (32–35°S and 58–62°W) on silty clay loam soil, located in the highlands within the Arroyo Dulce soil series (34). We chose four fields with a long cropping history (more than 15 yr since last pasture) and characterized by a low stability index (less than 20%), expressed as (100 × deteriorated soil mean-weighted diameter/pristine soil mean-weight diameter) of aggregates (16). Within each field we collected at random specimens of entire plants with similar height from two kind of sites: (i) continuously cultivated, and (ii) nondisturbed. Chemical and physical properties (Table 1) that define continuously cultivated soils and nondisturbed soils as deteriorated and nondeteriorated were previously determined (9).

Since all specimens were collected on the same day from fields in the same area, all environmental conditions were the same except for soil exploitation level. A voucher specimen of each sample was deposited (no. 24038) in the Herbarium of the Cátedra de Botánica, Facultad de Agronomía, UBA, Buenos Aires, Argentina.

Extraction. Aerial parts (stems and leaves) of *C. album* were separated from roots, and immediately submitted to drying conditions at 40°C, under ventilation, the same day that they were collected. The dry material was milled to a coarse powder prior to Soxhlet extraction with light petroleum ether. Four samples (100 g each) of dry plant material from each kind of soil were submitted to continuous extraction (300 mL). Each extract was evaporated *in vacuo* to dryness and successively percolated with *n*-hexane, methylene chloride, acetone, and methanol to give the corresponding subextracts. Further separation procedures included medium-pressure liquid chromatography (MPLC). All extracts, subextracts, and MPLC fractions were qualitatively analyzed by TLC and submitted to capillary GC and to GC–MS analysis.

Chromatographic conditions. TLC was performed on silica gel GF-254 (250 µm layers; Merck, Darmstadt, Germany) plates; high-performance TLC (HPTLC) was carried out on commercial plates (silica gel F-254, Merck). Mobile phases were as follows: (A) *n*-hexane/chloroform (95:5), (B) *n*-hexane/chloroform (6:4), and (C) light petroleum ether/ethyl ether/acetic acid (90:10:1). Spots were visualized under UV light at 254 nm and/or with sulfuric acid/acetic acid (1:1), with heating at 110°C for 5 min. Selective chromogenic reactions included bromothymol blue for lipids; anisaldehyde/sulfuric acid for steroids and terpenes; vanillin/sulfuric acid for higher alcohols and ketones; silver nitrate/ammonia for aldehydes; and silver nitrate/pyrogallol for acids.

TABLE 1
Chemical and Physical Indicators of Soil Quality[a]

Indicator (mean value)	Continuously cultivated soil (CC)	Nondisturbed soil (ND)
C_o (organic carbon), g kg^{-1}	20b	28a
N_t (total nitrogen), g kg^{-1}	2.1b	2.9a
P, mg kg^{-1}	17b	111a
B, mg kg^{-1}	0.3b	0.8a
Zn, mg kg^{-1}	1.9b	6.5a
SA_s (soil aggregate stability), mm	1.66c	0.31a
pH	6.2a	6.4a

[a]Within a row, means followed by a different letter show significant differences among soil types ($P < 0.05$).

MPLC was carried out under nitrogen pressure on silica gel H (Merck) columns, eluting with gradients of *n*-hexane/ethyl acetate (5:1 to 1:5); ethyl acetate; and gradients of ethyl acetate/methanol (49:1 to 1:5); main components were isolated, identified, and quantified.

GC was performed on a Hewlett-Packard 5890 gas chromatograph with a temperature program from 100 to 280°C at a rate of 15°C/min and then isothermally, by using a FID and a RSL-150 capillary column (50 m × 0.20 mm i.d. × 0.25 µm thickness; Alltech, Deerfield, IL) and nitrogen as carrier. Capillary GC–EI–MS analysis was performed at 70 eV on a Trio 2-VG quadrupole spectrometer, under programmed temperature from 100 to 280°C at a rate of 6°C/min. EI–MS data were processed using a Lab Base GC–MS data system; relative abundances (as percentages) were registered vs. retention times. Library matching was used to identify compounds tentatively. Comparing mass spectra with those of authentic commercial samples provided the final identification.

Statistical analysis. Statistical analysis was performed by means of an ANOVA, and mean values were compared by Tukey's test.

RESULTS

TLC analysis. Saturated hydrocarbons and waxes were detected as the main components in the *n*-hexane subextract, together with minor spots corresponding to terpenes and steroids (mobile phase A). Terpenoids, steroids, and aldehydes were detected as the main constituents of the dichloromethane subextract. The acetone subextract gave two major spots, characterized as aldehydes and alcohols; FA were also present. Alcohols and FA were the main components of the methanol subextract. MPLC confirmed these results and allowed the identification of minor components by GC–EI–MS and the isolation and quantification of the main ones. The contribution of different compounds to total dry matter was then calculated relative to those main compounds already quantified, by their relative abundances in the corresponding GC chromatogram.

Comparison of GC chromatograms (Fig. 1) of nonpolar extracts from aerial parts of *C. album* grown in continuously cultivated and in nondisturbed soils showed differences in the relative chemical composition of mainly hydrocarbons (Table 2). No major differences in the composition of the bulk lipid matrix were found, except for remarkably higher levels of long-chain hydrocarbons and related long-chain derivatives, along with lower relative levels of FA.

The EI–MS spectra [*m/z* (%)] of representative compounds are presented below.

(i) Tritetracontane (Peak 26, Table 2). 41 (14), 43 (49), 55 (25), 56 (17), 57 (100), 58 (6), 69 (20), 70 (12), 71 (68), 82

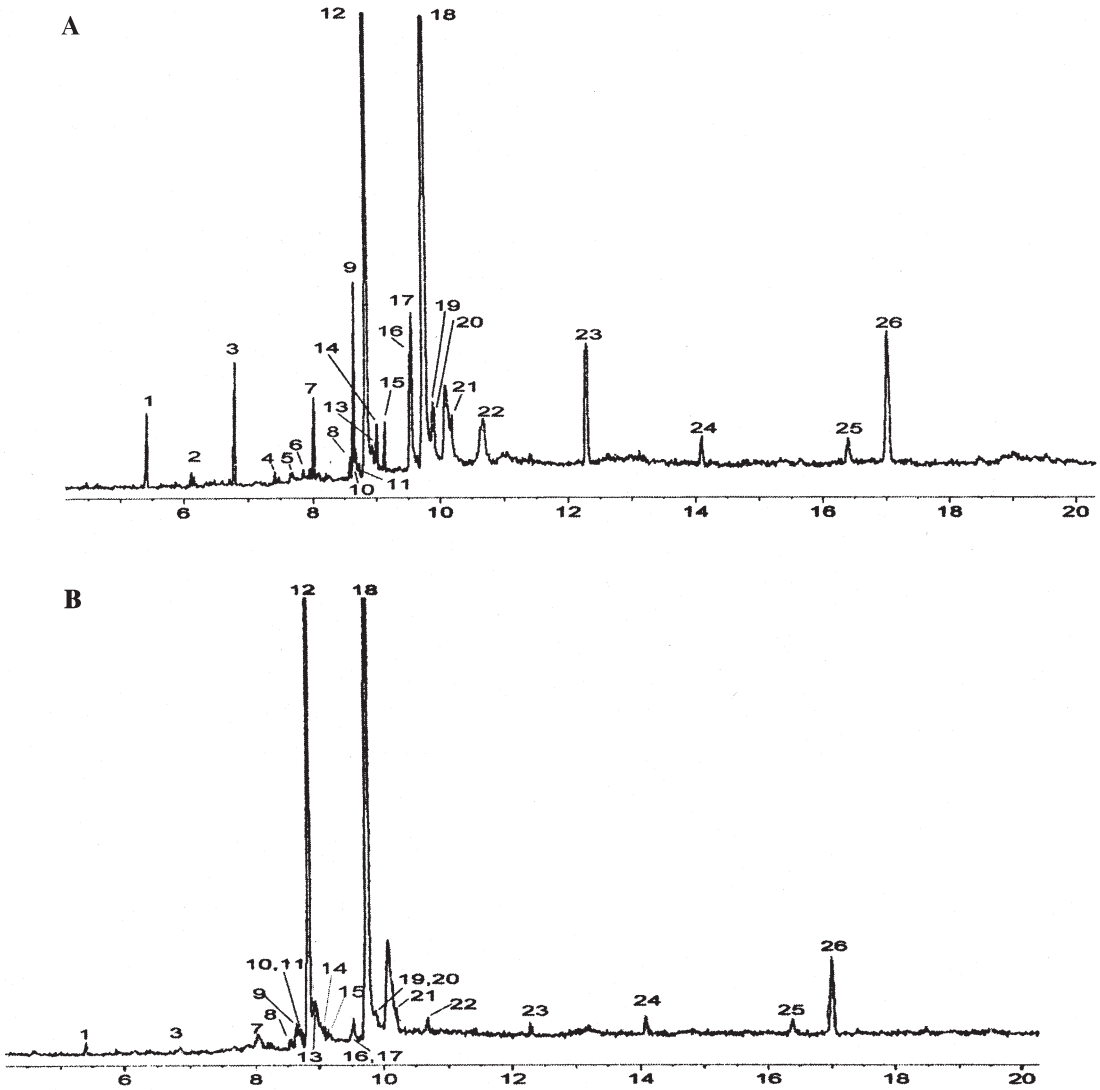

FIG. 1. Gas chromatograms of nonpolar extracts from aerial parts of *Chenopodium album* growing in continuously cultivated (A) and nondisturbed (B) soils.

(6), 83 (20), 84 (7), 85 (39), 97 (15), 98 (5), 99 (11), 111 (8), 113 (7), 127 (6), 141 (5).

(ii) Tetracontane (Peak 23, Table 2). 41 (10), 43 (49), 55 (19), 56 (11), 57 (100), 69 (13), 70 (9), 71 (77), 82 (5), 83 (16), 84 (6), 85 (48), 97 (15), 98 (5), 99 (19), 111 (9), 113 (8), 127 (13), 141 (10), 155 (6), 169 (5), 183 (5).

(iii) Pentatriacontane (Peak 22, Table 2). 41 (12), 42 (5), 43 (59), 55 (23), 56 (18), 57 (100), 69 (20), 70 (11), 71 (73), 83 (18), 84 (7), 85 (50), 97 (15), 98 (5), 99 (15), 111 (9), 112 (6), 113 (11), 125 (5), 126 (5), 127 (7), 141 (5), 492 (7).

(iv) n-*Nonacosane (Peak 21, Table 2).* 41 (19), 42 (5), 43 (79), 55 (23), 56 (14), 57 (100), 58 (5), 69 (18), 70 (12), 71 (74), 83 (17), 84 (8), 85 (53), 97 (12), 98 (5), 99 (20), 111 (7), 113 (15), 125 (5), 127 (11), 141 (10), 155 (6), 169 (5).

(v) n-*Heneicosane (Peak 15, Table 2).* 41 (26), 42 (5), 43 (61), 55 (20), 56 (11), 57 (100), 58 (6), 69 (11), 70 (10), 71 (72), 82 (10), 83 (10), 84 (6), 85 (57), 97 (7), 98 (6), 99 (20), 113 (10), 127 (7), 141 (5), M^+ 296 (1.8).

(vi) n-*Eicosane(Peak 14, Table 2).* 41 (43), 42 (10), 43 (90), 55 (30), 56 (17), 57 (100), 58 (5), 69 (15), 70 (10), 71 (68), 83 (10), 84 (9), 85 (50), 97 (7), 98 (5), 99 (9), 113 (8), 127 (6), 141 (5), M^+ 282 (7).

(vii) n-*Hexadecanal (Peak 24, Table 2).* 41 (89), 42 (22), 43 (100), 44 (40), 45 (15), 53 (5), 54 (14), 55 (63), 56 (27), 57 (85), 58 (5), 66 (8), 67 (34), 68 (40), 69 (37), 70 (19), 71 (30), 72 (6), 81 (24), 82 (53), 83 (31), 84 (10), 85 (11), 95 (20), 96 (28), 97 (18), 109 (8), 110 (8), 111 (6).

(viii) n-*Octadecanal (Peak 25, Table 2).* 41 (86), 42 (19), 43 (100), 44 (28), 45 (10), 53 (5), 54 (14), 55 (75), 56 (23), 57 (79), 58 (5), 66 (6), 67 (33), 68 (40), 69 (42), 70 (17), 71 (34), 72 (6), 81 (30), 82 (63), 83 (41), 84 (11), 85 (17), 95 (28), 96 (29), 97 (28), 98 (7), 99 (5), 109 (14), 110 (14), 111 (10), 123 (7), 124 (7), 137 (5), 138 (5).

DISCUSSION

The most remarkable feature in samples from continuously cultivated soils was a pronounced enhancement of long-chain

TABLE 2
Mean Contents (mg/g dry weight) of Nonpolar *Chenopodium album* Constituents from Plants Grown in Continuously Cultivated (CC) and Nondisturbed (ND) Soils

Peak no.[a]	Compound	CC (mean value)	ND (mean value)	*P*
1	*n*-Tetradecane	0.246 ± 0.077	0.034 ± 0.005	<0.001
2	*n*-Pentadecane	0.068 ± 0.0073	Trace[b]	
3	*n*-Hexadecane	0.286 ± 0.014	0.024 ± 0.004	<0.001
4	*n*-Heptadecane	0.052 ± 0.009	Trace	
5	2,6-Dimethylheptadecane	0.068 ± 0.012	Trace	
6	2-Methyloctadecane	0.040 ± 0.006	Trace	
7	*n*-Octadecane	0.213 ± 0.012	0.020 ± 0.003	<0.001
8	2,6,10,14-Tetramethylheptadecane	0.054 ± 0.007	0.013 ± 0.002	<0.001
9	Methyl *n*-hexadecanoate (methyl palmitate)	0.363 ± 0.038	0.053 ± 0.005	<0.001
10	Ethyl *n*-hexadecanoate (ethyl palmitate)	0.088 ± 0.011	0.037 ± 0.005	<0.001
11	Methyl *n*-octadecanoate (methyl stearate)	0.037 ± 0.003	0.019 ± 0.002	<0.001
12	*n*-Hexadecanoic acid (palmitic acid)	3.581 ± 0.264	4.385 ± 0.387	<0.001
13	*n*-Octadecanoic acid (stearic acid)	0.125 ± 0.011	0.205 ± 0.020	<0.001
14	*n*-Eicosane	0.213 ± 0.036	0.030 ± 0.005	<0.001
15	*n*-Heneicosane	0.207 ± 0.03	0.029 ± 0.004	<0.001
16	Methyl-(18:2) Δ9,12-*cis,cis*-octadienoate or methyl-9*Z*,12*Z* octadienoate or methyl linoleate	0.339 ± 0.022	0.016 ± 0.002	<0.001
17	Methyl (18:3) Δ9,12,15-*cis,cis,cis*-octadecatrienoate or methyl 9*Z*,12*Z*,15*Z*-octadecatrienoate or methyl linolenate	0.407 ± 0.025	0.076 ± 0.006	<0.001
18	(18:2) Δ9,12-*cis,cis*-octadienoic acid or 9*Z*,12*Z*-octadienoic acid or linoleic acid	4.076 ± 0.233	5.070 ± 0.301	<0.001
19	(18:2) Δ9,12-*cis,cis*-octadecadien-1-ol or 9*Z*,12*Z*-octadecadien-1-ol	0.205 ± 0.023	0.113 ± 0.011	<0.029
20	*n*-Octacosane	0.182 ± 0.078	0.055 ± 0.006	<0.001
21	*n*-Nonacosane	0.195 ± 0.032	0.099 ± 0.012	<0.001
22	*n*-Pentatriacontane	0.413 ± 0.080	0.047 ± 0.004	<0.001
23	*n*-Tetracontane	0.322 ± 0.025	0.040 ± 0.004	<0.001
24	*n*-Hexadecanal	0.146 ± 0.015	0.089 ± 0.005	<0.001
25	*n*-Octadecanal	0.157 ± 0.017	0.086 ± 0.004	<0.001
26	*n*-Tritetracontane	0.583 ± 0.033	0.351 ± 0.012	<0.001

[a]Numbers of the compounds refer to peaks in Figure 1.
[b]Traces (<0.01 mg/g dry weight).

linear hydrocarbons—such as hexadecane, eicosane, heneicosane, nonacosane, pentatriacontane, tetracontane, and tritetracontane—and methyl and ethyl esters derived from common FA, in comparison with samples from undisturbed soils. Smaller increments were found in long-chain aldehydes (*n*-hexadecanal and *n*-octadecanal). There was also an increment in the relative amount of unsaturated long-chain alcohols and branched long-chain hydrocarbons. Likewise, the relative proportion of FFA (palmitic, linoleic, and stearic acids) was diminished.

Long-chain hydrocarbons are ubiquitous in the waxy coating of leaves. Biosynthetically related to FA, they may play a role in plant disease resistance (35). The various biochemical steps of wax biosynthesis are known (36–38). The impact of variations in environmental parameters on the production of secondary metabolites by plants is well known (39–42). In response to biotic and abiotic stresses, plants produce more secondary metabolites. Higher levels of certain products of secondary metabolism, found under stress conditions, can negatively affect potential predators and may reflect natural selection, resulting in increased resistance to herbivores or to disease organisms and to future damage (40).

For example, water stress increases the levels of long-chain alkanes in cotton epicuticular waxes, *n*-tetratriacontane being the mayor wax constituent in leaves and *n*-triacontane in boll and bract waxes (43). Since many of these allelochemicals may possess multiple modes of action (20), their occurrence in higher levels under stress conditions can negatively affect potential predators. Changes in relative proportions of the different epicuticular wax components like FA and hydrocarbons may affect herbivory through their influence on oviposition (44). Differences in the proportions of *n*-alkanes, long-chain FA, esters, and aldehydes of *Brassica oleracea* epicuticular waxes found as a result of changes in growth conditions have also been related to the defense mechanisms of this organism (45).

The production of larger amounts of allelopathic compounds in a stressful environment can also be interpreted as an adaptive strategy to suppress the increase of competition for nutrients (42).

As a result of the overuse of agrochemicals, many herbivores, pathogens, and weeds have developed resistance to synthetic agrochemicals, making it necessary to find new methods of pest control based on natural products (20). The possible management of the chemical properties of weed species with potential biologic activities may help lessen and even revert agroecosystem damage (46).

ACKNOWLEDGMENTS

The research was financially supported by grants Proyecto de Investigación Plurianual 0619 (CONICET-Argentina) and Universidad de Buenos Aires. Ciencia y Técnica G047 (UBA–Argentina).

REFERENCES

1. Wink, M. (1993) Production and Application of Phytochemicals from an Agricultural Perspective, in *Phytochemistry and Agriculture* (van Beek, T.A., and Breteler, H., eds.), pp. 171–212, Proceedings of the Phytochemical Society of Europe, Vol. 34, Oxford University Press, Oxford.
2. Chou, C.H. (1999) Roles of Allelopathy in Plant Biodiversity and Sustainable Agriculture, *Crit. Rev. Plant Sci. 18*, 609–636.
3. Lafrance, P., and Giroux, I. (1999) Hydrogeologie et eaux souterraines au Québec, *Hydrogeologie (France) 4*, 43–50.
4. Steen, R.J.C.A., Leonards, P.E.G., Brinkman, U.A.T., Barcelo, D., Tronczynski, J., Albanis, T.A., and Cofino, W.P. (1999) Ecological Risk Assessment of Agrochemicals in European Estuaries, *Environ. Toxicol. Chem. 18,* 1574–1581.
5. DeSutter, T.M., Clay, S.A., and Clay, D.E. (1998) Atrazine, Alochlor, and Total Inorganic Nitrogen Concentrations of Winter Wind-Eroded Sediment Samples, *Agr. Wastes 33*, 683–691.
6. Pereira, W.E., and Hostettler, F.D. (1993) Nonpoint Source Contamination of the Mississippi River and Its Tributaries by Herbicides, *Environ. Sci. Technol. 27*, 1542–1552.
7. Zhou, J.L., Maskaoui, K., Qiu, Y.W., Hong, H.S., and Wang, Z.D. (2001) Polychlorinated Biphenyl Congeners and Organochlorine Insecticides in the Water Column and Sediments of Daya Bay, *China Environ. Pollut. 113*, 373–384.
8. Karlen, D.L., Gardner, J.C., and Rosek, M.J. (1998) The Soil Quality Framework for Evaluating the Impact of CRP, *J. Prod. Agric. 11,* 56–60.
9. Maddonni, G.A., Urricarriet, S., Ghersa, C.M., and Lavado, R.S. (1999) Assessing Soil Quality in the Rolling Pampa, Using Soil Properties and Maize Characteristics, *Agron. J. 91,* 280–287.
10. Hall, A.J., Rebella, C.M., Ghersa, C.M., and Culot, J.P. (1992) Field-Crop Systems of the Pampas, in *Ecosystems of the World: Field Crop Ecosystems* (Pearson, C.J., ed.), pp. 413–450, Elsevier, Amsterdam.
11. Ghersa, C.M., and Martínez-Ghersa, M.A. (1991) Cambios ecológicos en los agroecosistemas de la Pampa Ondulada, Efectos de la introducción de la soja, *Cienc. Invest. 2,* 182–188.
12. Michelena, R.O., Irurtia, C.B., Vavruska, F.A., Mon, R., and Pittaluga, A. (1989) Degradación de suelos en el Norte de la Región Pampeana, *INTA Publ. Tec. 6*, Centros regionales de Buenos Aires Norte, Córdoba, Entre Ríos y Santa Fé, Proyecto de Agricultura Conservacionista.
13. Maddonni, G.A., Iglesias Pérez, M.E., Cárcova, J., and Ghersa, C.M. (2000) Maize Flowering Dynamic in Soils with Contrasting Agricultural History, *Maydica 44*, 141–147.
14. de la Fuente, E.B., Suárez, S.A., Ghersa, C.M., and León, R.J.C. (1999) Soybean Weed Community: Relationship with Cultural History and Crop Yield, *Agron. J. 91,* 234–241.
15. Ghersa, C.M., and León, R.J.C. (1999) Successional Changes in Agroecosystems of the Rolling Pampa, in *Ecosystems of Disturbed Ground* (Walker, L.R., ed.), pp. 487–502, Elsevier, Amsterdam.
16. Gil, A., de la Fuente, E.B., Lenardis, A.E., López Pereira, M., Suárez, S.A., Bandoni, A., van Baren, C., Di Leo Lira, P., and Ghersa, C.M. (2002) Coriander Essential Oil Composition from Two Genotypes Grown in Different Environmental Conditions, *J. Agric. Food Chem. 50*, 2870–2877.
17. Altieri, M.A., and Whitcomb, W.H. (1978) The Potential Use of Weeds in the Manipulation of Beneficial Insects, *HortScience 14*, 12–18.
18. Su, H.C.F. (1991) Toxicity and Repellency of Chenopodium Oil to Four Species of Stored-Product Insects, *J. Entomol. Sci. 26*, 178–182.
19. Qasem, J.R. (1995) Allelopathic Effects of *Amaranthus retroflexus* and *Chenopodium murale* on Vegetable Crops*, Allelopathy J. 2,* 49–66.
20. Quarles, W. (1992) Botanical Pesticides from *Chenopodium*? *IPM Practitioner 16*, 1–11.
21. De Prado, R., Lopez-Martinez, N., and Giménez-Espinoza, R. (1997) Herbicide-Resistant Weeds in Europe: Agricultural, Physiological and Biochemical Aspects, in *Weed and Crop Resistance to Herbicides* (De Prado, R., Jorrin, J., and García-Torres, L., eds.), pp. 17–27, Kluwer, Dordrecht.
22. Qasem, J.R., and Hill, T.A. (1989) Possible Role of Allelopathy in the Competition Between Tomato, *Senecio vulgaris* L. and *Chenopodium album* L., *Weed Res. 29,* 349–356.
23. Bera, B., Ganguly, S.N., and Mukherjee, K.K. (1992) Growth Retarding Compounds in the Seeds of Diploid Cytotypes of *Chenopodiun album*, *Fitoterapia 63*, 364–366.
24. Reinhardt, C.F., Meissner, R., and Van Wyk, L.J. (1997) Allelopathic Effects of *Chenopodium album* L. and *Chenopodium polyspermum* L. on Another Weed and Two Crop Species, *South Afr. J. Plant Soil 14*, 165–168.
25. Dutt, S., Balasubrahmanyam, A., and Lodha, M.L. (2000) Purification and Partial Characterization of Antiviral Proteins from *Chenopodium album* L. Leaves, *J. Plant Physiol. 156*, 808–810.
26. Navrezova, N., Agzamova, M., Stepanichenko, N.N., and Makhsudova, B. (1984) Action of the Flavonoids Quercetin-3-rutinoside and Kaempferol-3,7-dirhamnoside on the Biosynthesis of Melanin in *Verticillium dahliae, Chem. Nat. Compd. 22,* 229–230.
27. Tabil, M.A., and Walia, K.K. (1997) Control of Root-Knot Nematode, *Meloidogyne incognita* by Amendment of Nursery Soil with *Chenopodium* Species, *Ann. Plant Protect. Sci. 5*, 61–65.
28. Lavaud, C., Voutquenne, L., Bal, P., and Pouny, I. (2000) Saponins from *Chenopodium album, Fitoterapia 71*, 338–340.
29. Gonzalez, J.A., Gallardo, M., and De Israilev, L.A. (1998) Leaf Flavonoids in *Chenopodium hircinum* Schrad. and *Chenopodium album* L., *Phyton (Buenos Aires) 63*, 279–281.
30. Prakash, D., Nath, P., Pal, M., and Pashupati, N. (1993) Composition, Variation of Nutritional Contents in Leaves, Seed Protein, Fat and Fatty Acid Profile of *Chenopodium* Species, *J. Sci. Food Agr. 62*, 203–205.
31. Younes, M.E., Amer, M.S., and El Messallami, A.D.E. (1996) Phytochemical Examination of the Leaves of the Egyptian *Zyziphus spina christi* "Nabc," *Bull. Nat. Res. Centre, Cairo 21*, 35–40.
32. Archana, P., Shukla, Y.N., Tripathi, A.K., and Pande, A. (1995) Lipid Constituents from *Stellaria media, Phytochemistry 39*, 709–711.
33. Aarts, M.G.M., Keijzer, C.J., Stiekema, W.J., and Pereira, A. (1995) Molecular Characterization of the CER1 gene of *Arabidopsis* Involved in Epicuticular Wax Biosynthesis and Pollen Fertility, *Plant Cell 7*, 2115–2127.
34. *Carta de suelos de la República Argentina* [*Argentine Republic Soil Chart*]; hoja 3560-2 Rojas, 3560-3 Salto; ISAG: Buenos Aires, Argentina, 1974, Instituto Nacional de Tecnología Agropecuaria, INTA.
35. Harborne, J.B. (1998) *Phytochemical Methods*: *A Guide to Modern Techniques of Plant Analysis*, 3rd edn., pp. 170–174, Chapman & Hall, London.
36. von Wettstein-Knowles, P. (1979) Genetics and Biosynthesis of Plant Epicuticular Waxes, in *Advances in the Biochemistry and Physiology of Plant Lipids* (Appelqvist, L.A., and Liljenberg, C., eds.), pp. 1–26, Elsevier/North Holland Biochemical Press, Amsterdam.
37. Bianchi, A., Bianchi, G., Avato, P., and Salamini, F. (1985)

Biosynthetic Pathways of Epicuticular Wax of Maize as Assessed by Mutation, Light, Plant Age and Inhibitor Studies, *Maydica 30,* 179–198.
38. von Wettstein-Knowles (1994) Biosynthesis and Genetics of Waxes, in *Waxes: Chemistry, Molecular Biology and Functions* (Hamilton, R.J., ed.), pp. 91–129, Oily Press, Dundee, Scotland.
39. Einhellig, F.A. (1989) Interactive Effects of Allelochemicals and Environmental Stress, in *Phytochemical Ecology: Allelochemicals, Mycotoxins and Insect Pheromones and Allomones* (Chou, C.H., and Waller, G.R., eds.), pp. 101–117, Institute of Botany, Academia Sinica Monograph Series no. 9, Taipei, ROC.
40. Gershenzon, J. (1984) Changes in the Levels of Plant Secondary Metabolites Under Water and Nutrient Stress, *Rec. Adv. Phytochem. 18*, 273–321.
41. Einhellig, F.A. (1996) Interactions Involving Allelopathy in Cropping Systems, *Agron. J. 88,* 886–893.
42. Tang, C.S., Cai, W.F., Kohl, K., and Nishimoto, R.K. (1995) Plant Stress and Allelopathy in *Allelopathy: Organisms, Processes and Applications* (Inderjit, Dakshini, K.M.M., and Einhellig, F.A., eds.), pp. 142–157, ACS Symposium Series No. 582, American Chemical Society, Washington, DC.
43. Bondada, B.R., Oosterhuis, D.M., Murphy, J.B., and Kim, K.S. (1996) Effect of Water Stress on the Epicuticular Wax Composition and Ultrastructure of Cotton (*Gossypium hirsutum* L.) Leaf, Bract and Boll, *Environ. Exp. Bot. 36,* 61–69.
44. Parr, M.J., Tran, B., Simmonds, M.S.J., Kite, G.C., and Credland, P.F. (1998) Influence of Some Fatty Acids on Oviposition by the Bruchid Beetle, *Callosobruchus maculatus, J. Chem. Ecol. 24,* 1577–1593.
45. Shepherd, T., Robertson, G.W., Griffiths, D.W., Birch, A.N.E., and Duncan, G. (1995) Effects of Environment on the Composition of Epicuticular Wax from Kale and Swede, *Phytochemistry 40*, 407–417.
46. Chou, C.H. (1995) Allelopathy and Sustainable Agriculture in *Allelopathy: Organisms, Processes and Applications* (Inderjit, Dakshini, K.M.M., and Einhellig, F.A., eds.), pp. 211–223, ACS Symposium Series No. 582, American Chemical Society, Washington, DC.

[Received July 19, 2002, and in revised form April 3, 2003; revision accepted April 14, 2003]