Allelopathic potential of *Medicago arborea*, a Mediterranean invasive shrub

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Summary. Biological invasions are nowadays a major problem in ecology. Allelopathy has been shown to be involved in such invasions, but this mechanism has been little studied in France. The objectives of this study were to evaluate the allelopathic potential of *Medicago arborea*, an invasive species in the French Mediterranean region. Foliar extracts were tested on three target species (*Lactuca sativa*, *Lepidium sativum* and *Linum strictum*). We showed that *Medicago arborea* has high allelopathic potential to affect the growth and germination of other species. Yellow flax (*Linum strictum*), native to the invaded area, was the most sensitive of the tested plant species to foliar extracts of *Medicago arborea*. Our study pointed out the role of allelopathy in processes leading to biological invasion, and more generally in population dynamics.

Key words: allelopathy – biological invasion – population dynamics – biodiversity – phenolic compounds – *Medicago arborea* (Fabaceae, Fabales, Rosidae).

Introduction

Globalisation of the world trade market has caused widespread introduction of exotic species at a worldwide scale. Introduced species make up a large part of the flora and fauna in most regions of the world (Mack and D'Antonio, 1998), and introduction of non-native species can either be rewarding or a plague to the society (Lambinon, 1997; Ewel *et al.*, 1999, Vivanco *et al.*, 2004). Numerous introduced species play fundamental roles in the economy and culture of many countries (*e.g.* food, medical industry and horticulture). However, some of these species are spreading into the wild where they become invasive and have profound negative impacts on native ecosystems (Williamson, 1996). Successful invasions often results in irreversible homogenisation of plant communities (Wiser *et al.*, 1998). Biological invasions are thought to be the second largest threat, after the destruction or fragmentation of habitats, to sustaining biodiversity at a global scale (D'Antonio and Vitousek, 1992; Williamson, 1996, 1999).

The circum-Mediterranean region is an important pool of global biodiversity (Myers in Médail and Quézel, 1997), apparently due to specific climatic conditions, the diverse origin of the flora, habitat heterogeneity, and geological, paleogeographical and historical factors. For example, in south of France, there are 215 endemic taxa, which represent 7.2 % of the regional flora (Médail and Verlaque, 1997). Preservation of this biological heritage is important.

Current research on biological invasion has focused on (i) characteristics of habitats most likely to be invaded (Orions, 1986; Crawley, 1987; Huenneke *et al.*, 1990; Harrington, 1991; Bruke and Grime, 1996; Wiser *et al.*, 1998), including the role of perturbation for facilitating invasions (Hobbs and Huenneke, 1992; D'Antonio *et al.*, 1999), (ii) processes of invasion (Vivrette and Muller, 1977; Zedler and Scheid, 1998; D'Antonio, 1993; Vila and D'Antonio, 1998), including characteristics (ecological, physiological and biological attributes) of species most likely to be invasive (Hulst *et al.*, 1987; Noble, 1989; Albert, 1995; Binggeli, 1996; Rejmanek, 1996; Reichard, 1997), and to a lesser extent (Alvarez, 1999) (iii) impacts of invasive species on ecosystems. Many studies have been carried out on competition, while few studies have looked at allelopathic potential among invasive species and its role in the invasion process (Bais *et al.*, 2003; Hierro and Callaway, 2003).

Among vascular plants, almost all allelochemicals are secondary metabolites, and their impacts on ecosystems have been studied for several years (Rice, 1984). These studies have permitted to anticipate variations in their toxicity with regard to their ecological and seasonal context. It has been shown that allelopathic component play an important role in the regulation of plant diversity (Chou, 1999).

We chose to study *Medicago arborea* L. (Fabaceae, Fabales). Its widespread distribution is a result of human cultivation and use as ornamental plant or to prevent soil erosion (Andreu *et al.*, 1998). This African species has spread mainly along the coast in the vicinity of houses in residential areas where it used to be planted, but it has also been recorded as invasive in the Mediterranean region. The aim of this study is to analyse allelopathic potential of this introduced plant species, in order to understand the role of

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this phenomenon in the processes of succession and on the regulation of biodiversity of plant communities.

Material and methods

Plant Material. M. arborea, tree medic, originating from Africa, is a woody perennial plant species that can reach 2 m in height. Sampling of plant material was made along the coast in the municipality of Martigues (Bouches-du-Rhône, south-eastern France). Leaves from five individuals of similar age were sampled at all heights in dense, almost mono-specific, stands. Sampling was carried out in October before leaf fall. Leaves were frozen and then oven-dried at 30 °C until constant weight.

Chemical Analyses. Aqueous extracts of compounds were prepared by soaking 249.4 g fresh leaves in 1,150 g distilled water for 48 hours in darkness at 4 °C. The obtained solution was filtered and freeze-dried to give 11.35 g of yellow powder. A mass of 200 mg of the powder was dissolved in 25 ml water (HPLC quality – Millipore-St-Quentin, France) and the solution was extracted three times with 25 ml ethyl acetate (SDS-Peypin, France). The three organic fractions were gathered and concentrated to dryness with rotary evaporator. The resulting residue was suspended in 1 ml of methylene chloride (SDS-Peypin, France) and transferred to a 5 ml vial. The procedure was repeated three times. The collected solution was evaporated to dryness under a stream of helium, and 500 µl of methylene chloride was then once again added, then evaporated, to remove residual water.

For GC-MS analysis, 200 µl of Derivative reagent, N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1 % trimethylchlorosilane (TMCS) (purchased by Fluka-Sigma Aldrich), was used and an equal volume of acetonitrile (SDS-Peypin, France) was added to produce trimethylsilyl derivatives. The solution was incubated at 70 °C for 1 hour.

Analyses were performed using a Hewlett-Packard GC6890 coupled to a HP 5973N Mass Detector. Separation of the analytes was achieved by using a HP5 MS capillary column of 30 m length × 0.25 mm I.D. × 0.25 µm film thickness (J&W Scientific). Sample volumes of 2µl were injected in a splitless mode for 1min with an ALS 7683 Automatic Injector. Purge flow was set to 30ml/min. Helium (99.995 %) was used as carrier gas. Initial head pressure was kept at 8.22 psi and a constant flow of 1 ml/min was set throughout the run. Initial oven temperature was programmed at 70 °C, and was gradually increased to 220 °C at a rate of 3 °C/min, where it remained constant for 10min. Injector temperature and MSD transfer line heater were kept constant at 250 and 280 °C, respectively. Mass spectrometer parameters for EI mode were: ion source, 230 °C; MS quadrupole, 150 °C; electron energy, 70 eV; electron multiplier energy, 1150 V; acquisition in scan mode from 40 to 500 amu.

Bioassays. Allelochemicals are released into the environment through 4 principal pathways: decomposition of litter, root exudates in the soil, vaporisation into the air, and rain and dew transfering leaf compounds to the soil (Rice, 1984). Some authors have shown that water-soluble compounds are, probably, those compounds most likely to be involved in allelophathy (Vyvyan, 2002). We have chosen to approach natural leachates in this study by testing foliar aqueous extracts.

A base solution of 20 g l^{-1} was prepared from 4 g of foliar tissue and 200 ml of deionised water (Molina *et al*., 1991; Bong-Seop, 1992; Vyvyan, 2002). The extract was left for 27 hours in a refrigerator (4 °C ± 2 °C), shaken occasionally, and was then filtered through Whatman No. 4 paper. This base solution was then used for preparing dilutions of 5 g l^{-1}, 10 g l^{-1}, and 20 g l^{-1}.

The allelopathic potential of leaves of *Medicago arborea* was tested on seeds from three species: garden cress (*Lepidium sativum* L., Brassicaceae, Brassicales), lettuce (*Lactuca sativa* L. c.v. *battavia,* Asteraceae, Asterales), and yellow flax (*Linum strictum* L., Linaceae, Linales). These species are known for their sensitivity to allelopathic substances. Lettuce and garden cress are frequently used for bioassays (Heisey, 1990; Lawrence *et al.*, 1991). Yellow flax was chosen because it naturally grows in Mediterranean ecosystems, S France, and because it is sensitive to allelochemicals (Deleuil, 1950). Seeds of the first two species were bought in local commerce while seeds of the latter species were obtained through the botanical garden of Marseille.

Trials were carried out using glass Petri dishes (9 cm diameter) and Whatman paper No. 4. Twenty seeds were placed in each Petri dish, to which 2 ml of foliar extract was added, except for controls that received 2 ml deionised water. Seeds were sprayed every 24 hours with the original test extract to avoid dessication and to reflect natural conditions during rain: target plants are assumed to be in contact with allelochemicals, or with deionised water in the case of controls. For each dose, five replicates were used.

Trials were carried out at room temperature (approximately 20 °C and natural photoperiod). Germination rate and response curve, as well as the growth of seedlings (length of the radicle and hypocotyl, and total length) were measured.

Statistical Analyses. Comparison of germination rate between treatments was tested using a Chi-square test (Scherrer, 1984). Differences in mean length (radicle, hypocotyl and total length) were tested using analyses of variance (one-way ANOVA). Prior to analyses, data was tested for normality and homogeneity of variance by Shapiro-Wilks' and Bartlett's tests, respectively. Statistical analyses were carried out using Statgraphics® (version 2.1).

Results

Chemical Analyses. Analyses of *M. arborea* leaves demonstrated the presence of 4-hydroxybenzoic, vanillic and p-coumaric acids. Salicylic acids was also identified. Other, non-phenolic, acids such as succinic, palmitic, azelaic, lactic, dehydroabietic acids were as well identified (Table 1).

Effects on Germination. Germination rate varied between 48 and 99%, depending on species and dose (Fig. 1). Yellow flax was the only target species that had lower germination rate in the presence of foliar extracts of *M. arborea*, and this was significant only at highest dose (20 g l^{-1}; Khi 2 test, $p < 0.05$). Additionally, the germination rate of yellow flax seeds was lower at the highest dose (complete germination was not reached until 12 days after initiation of the experiment, Fig. 2).

Effects on Growth. Foliar extracts of *M. arborea* strongly reduced growth of seedlings for all three target species (One-way Anova, $p < 0.05$; Fig. 3). The inhibition of growth was particularly pronounced for extracts at doses 10 and 20g l^{-1}. It must further be emphasised that the reduction in growth was simultaneously observed on hypocotyl and radicle only at the highest dose, and this was consistent for all three target species (One-way Anova, $p < 0.05$; Fig. 3). At lower doses, inhibition of growth mainly affected the radicle. The effect of extract solutions on the radicle was particularly important for *L. strictum* and *L. sativa*. Indeed, necrosis was observed for the entire radicle at high doses (Fig. 3). It must also be underlined that a stimultating effect on the growth of hypocotyl of *L. sativum* was observed at low doses (5 g l^{-1}) (One-way Anova, $p < 0.05$; Fig. 3).

Discussion

Several authors have shown that invasive plants often form dense, mono-specific stands in their new habitats (Bais *et al.*, 2003) though it this density is quite rare in the natural environments of these same species. Such monocultures

Table 1 Chemical compounds extracted from *Medicago arborea* leaves. RT: retention time (minutes) of compounds in the column. Surface of the peaks in the chromatogram is expressed as a percentage

RT	COMMON NAME	SCIENTIFIC NAME	Surf (%)	CAS
	Aliphatic acids			
7.47	Lactic acid	Propanoic acid, 2-hydroxy-	2.0	50-21-5
9.18	2-Hexenoic acid	2-Hexenoic acid	0.5	1191-04-4
13.92	Succinic acid, ethyl ester	Butanedioic acid, monoethyl ester	< 0.5	1070-34-4
16.74	Succinic acid	Butanedioic acid	12.1	110-15-6
22.16	Capric acid	Decanoic acid	< 0.5	334-48-5
27.29	β-Phenyllactic acid	Benzenepropanoic acid, α-hydroxy-	< 0.5	828-01-3
29.55	Lauric acid	Dodecanoic acid	< 0.5	143-07-7
34.91	Azelaic acid	Nonanedioic acid	2.9	123-99-9
36.39	Myristic acid	Tetradecanoic acid	1.4	544-63-8
42.70	Palmitic acid	Hexadecanoic acid	3.2	57-10-3
48.47	Stearic acid	Octadecanoic acid	1.3	57-11-4
	Aliphatic alcohols			
15.43	Glycerol	1,2,3-Propanetriol	2.2	56-81-5
	Bases			
17.73	2,4-Pyrimidinediol	2,4-Pyrimidinediol	4.0	51953-14-1
20.28	2,4-Pyrimidinediol, 5-methyl-	2,4-Pyrimidinediol, 5-methyl-	2.5	80289-22-1
	Phenolic alcohols			
12.93	Phenylethyl alcohol	Benzeneethanol	0.7	60-12-8
21.44	α-hydroxy-o-cresol	Benzenemethanol, 2-hydroxy-	< 0.5	90-01-7
26.65	α-hydroxy-p-cresol	Benzenemethanol, 4-hydroxy-	2.5	623-05-2
29.32	Vanillic alcohol	Benzenemethanol, 4-hydroxy-3-methoxy-	< 0.5	498-00-0
	Phenolic aldehydes			
23.10	β-Resorcylic aldehyde (or Gentisaldehyde)	Benzaldehyde, 2,4-dihydroxy- (or Benzaldehyde, 2,5-dihydroxy-)	3.9	95-01-2 1194-98-5
25.05	Vanillin	Benzaldehyde, 4-hydroxy-3-methoxy-	< 0.5	121-33-5
	Phenolic acids and esters			
24.40	Salicylic acid	Benzoic acid, 2-hydroxy-	5.3	69-72-7
13.69	Benzoic acid	Benzoic acid	2.8	65-85-0
27.91	β-Resorcylic acid, methyl ester (or Gentisic acid, methyl ester)	Benzoic acid, 2,4-dihydroxy-, methyl ester (or Benzoic acid, 2,5-dihydroxy-, methyl ester)	2.5	2150-47-2 2150-46-1
28.71	4-Hydroxybenzoic acid	Benzoic acid, 4-hydroxy-	1.4	99-96-7
29.20	4-Hydroxybenzeneacetic acid	Benzeneacetic acid, 4-hydroxy-	< 0.5	156-38-7
33.76	Vanillic acid	Benzoic acid, 4-hydroxy-3-methoxy-	2.0	121-34-6
34.42	β-Resorcylic acid (or Gentisic acid)	Benzoic acid, 2,5-dihydroxy- (or Benzoic, 2,4-dihydroxy-)	< 0.5	89-86-1 490-79-9
38.39	Syringic acid	Benzoic acid, 3,5-dimethoxy-4-hydroxy-	< 0.5	530-57-4
	Cinnamic acids			
34.52	p-Coumaric acid	2-Propenoic acid, 3-(4-hydroxyphenyl)-	2.4	7400-08-0
	Others			
52.49	Dehydroabietic acid	1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-(1R,4aS,10aR)-	3.3	1740-19-8
21.36	2,3-Dihydroxybutyrolactone	2(3H)-furanone, dihydro-3,4-dihydroxy	< 0.5	13092-55-2
32.09	Non identified	m/z=303	7.2	

suggest that unusually strong interactions may be occurring, such as allelopathy, during the establishment of invasive plant species, in addition to competition for environmental resources (Abdul-Wahab and Rice, 1967; Vaughn and Berhow, 1999; Callaway and Aschehoug, 2000, Ridenour and Callaway, 2001).

Medicago species are known to contain water soluble compounds such as phenolic acids and derivatives (Xuan *et al.*, 2005), and terpenoids (Miller, 1996) that are allelopathic to other species (Oleszek *et al*., 1999, Chon *et al*., 2002). In this study, we chose to focus our analysis on low molecular weight compounds.

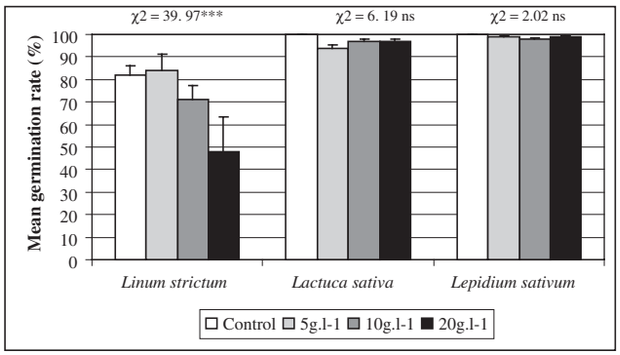

Fig. 1 Mean germination rate of the tree target species (confidence interval at 95 %) according to the dose of the extract of *Medicago arborea*. Results of Chi-Square test are also presented (χ^2 value and p: *** $p < 0.001$; ns: non-significant)

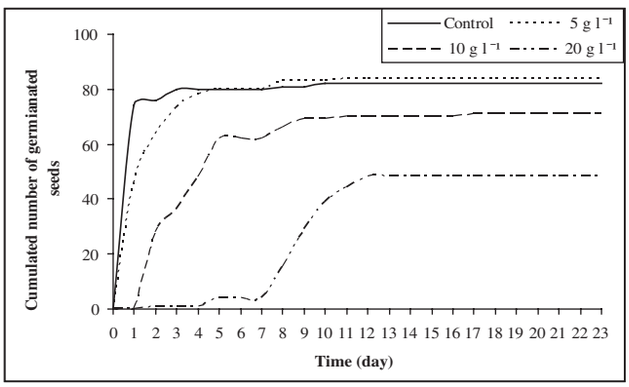

Fig. 2 Germination curve of *Linum strictum* seeds according to the dose of the extracts of *Medicago arborea*.

Phenolic compounds, found in this study, such as p-coumaric, 4-hydroxybenzoic and, vanillic acid, had already been detected in leaves of *M. arborea* (Torck and Pinkas, 1980). All these compounds may be involved in potential allelopathic effects of this species. Many phenolic compounds appear to have allelochemical effects (Rice, 1984; Bong-Seop, 1992). Furthermore, compounds such as dihydroabietic or salicylic acid have been described as allelochemicals (Quayyum *et al.*, 1999; Chung *et al.*, 2000). Leaves of *M. arborea* contain thus compounds that could have, after being released in the environment as leachates or litter exudates, allelopathic effects on other plants.

Our results show that leaf extracts of *M. arborea* have a noticeable allelopathic effect on the different tested species. Even if bioessay on cress and lettuce do not demonstrate the importance of allelopathy in invasions, the use of these species shows the possible mechanism by which *Medicago* competes with natives. Moreover, the results concerning yellow flax, which grows in the study area, have more ecological significance.

In allelopathy studies, 10 % extracts are commonly used (Rutherford et Powrie, 1993; Laterra et Bazzalo, 1999). This concentration of allelochemicals is probably higher than those found in fields, but it reveals an allelopathic potential from the donor plant (Bong-Seop, 1992). Different stages of the target species' life cycle were affected: germination (rate and response curve) as well as growth, particularly for yellow flax (species growing naturally in the environment). In this way, *M. arborea* could, through compounds present in their leaves, limit the establishment of other species by reducing interspecific competition (Reigosa *et al.*, 2000), or by affecting the growth of species already present in the environment. In particular, phytotoxic compounds released by *M. arborea* gave raise to, at high concentrations, a total necrosis in the radicle of the three target species. This

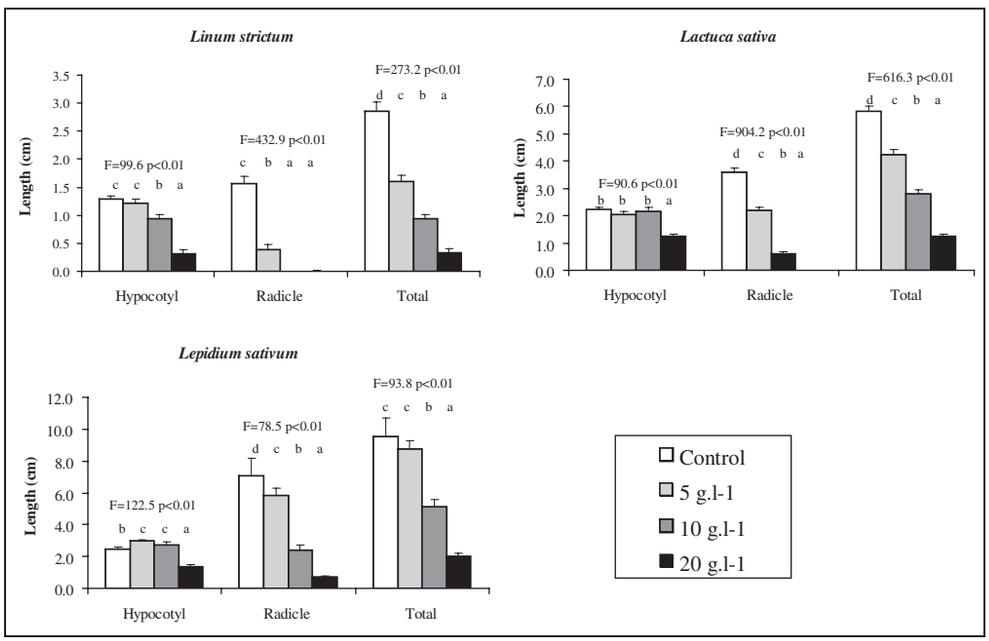

Fig. 3 Mean of radicle, hypocotyl and total length (confidence interval at 95 %) of *Linum strictum, Lactuca sativa, Lepidimum sativum* as a function of tested dose. Results of Anova tests (F- and p-value) and post-hoc tests on length are also presented. Values not differing at 5 % are denoted with the same letter

allelopathic damage could affect the competitiveness of plants. Resource competition and allelopathy should be considered together as processes that simultaneously affect the growth and population dynamics of other species (Nilsson 1996, Ridenour and Callaway, 2001). Their relative importance can vary depending on species and ecological context (Callaway and Aschehoug, 2000; Hierro and Callaway, 2003) such as the invasion by exotic species. Callaway and Aschehoug (2000) have by the way shown that the advantage of an invasive species over a native species appears to be due to root exudates and their effects on competition for resources. Such competitive mechanisms, that are not naturally present in the habitats invaded by exotic species, could interact with inherently coevolved interactions among long-associated native species (Callaway and Aschehoug, 2000). Allelopathy could thus play a more important role in the invasion process when the plants present in the colonised environment appear to be more sensitive to allelochemicals than the invasive species (Williamson, 1990). It has been shown that not only plants but also soil micro-organisms could possess an inherent resistance against allelochemicals in their natural environment while this resistance is not effective against invading exotic species (Vivanco *et al.*, 2004). In the same way, our results showing a high sensitivity of the species naturally present in the environment (*Linum structum*) towards the allelochemicals of *M. arborea*, compared to the commercialised species, could be due to lacking resistance against aggressive mechanisms involved in the invasion of this exotic species.

Our study shows that *M. arborea* possesses allelopathic potential. Allelopathic potential of *M. arborea* could favour its establishment in the environment that it colonises, whether it is limiting interspecific competition or access to resources, which could result in profound modifications of the colonised environments' biodiversity.

Acknowledgments

We would like to thank Dr Régine Verlaque for her help in this study and to Anders Mårell for correcting the English. We also like to thank Dr Virginie Baldy, Dr Nathalie Korboulewsky and the two anonymous referees for their comment and valuable criticism.

References

Abdul-Wahab AS, Rice EL (1967) Plant inhibition by Johnson grass and its possible significance in old-field succession Bull Torrey Bot Club 94: 486–497

Albert ME (1995) Portrait of an Invader II: The Ecology and Management of *Carpobrotus edulis* CalEPPC News Spring 1995

Alvarez ME (1999) Community level consequences of a biological invasion: Effects of a non-native vine on three plant communities MS Sonoma State University.

Andreu V, Rubio JL, Gimeno-Garcia, E, Llinares JV (1998) Testing three Mediterranean scrub species in runoff reduction and sediment transport Soil and Tillage Research 45: 441–454

Bais HP, Vepachedu R, Gilroy S, Callaway RM, Vivanco JM (2003) Allelopathy and exotic plants: from genes to invasion Science 301: 1377–1380

Binggeli P (1996) A taxonomic, biogeographical and ecological overview of invasive woody plants J Veg Sci 7: 121–124

Bong-Seop K (1992) Effect of pine allelochemicals on selected species in Korea. Pp. 204–241 *in* Rizvi SJH, Rizvi V (eds) Allelopathy: Basic and applied aspects. GB-London: Chapman & Hall publ

Burke MJW, Grime JP (1996) An experimental study of plant community invisibility Ecology 77: 776–790.

Callaway RM, Aschehoug, ET (2000) Invasive plants versus their new and old neighbors: a mechanism for exotic invasion Science 290: 521–523

Chon SU, Choi SK, Jung S, Jang HG, Pyo BS, Kim SM (2002) Effect of alfalfa leaf extracts and phenolic allelochemicals on early seedling growth and root morphology of alfalfa and barnyard grass Crop protection 21: 1077–1082

Chou CH (1999) Roles of Allelopathy in Plant Biodiversity and Sustainable Agriculture Plant Sci 18(5): 609–636

Crawley MJ (1987) What makes a community invasible? Pp 429–453 Gray AJ, Crawley MJ, Edwards PJ (eds) Colonizers, succession and stability. GB-Oxford: Blackwell Scientific Publications

Chung IM, Seigler D, Miller DA, Kyung SH (2000) Autotoxic coumpound from fresh Alfalfa leaf extracts: identification and biological activity J Chem Ecol 26(1): 315–326

D'Antonio CM, Dudley TL, Mack M (1999) Disturbance and biological invasions: Direct effects and feedbacks *in* Walker L (ed) Ecosystems of Disturbed Ground Amsterdam: Elsevier Press.

D'Antonio CM (1993) Mechanisms controlling invasion of coastal plant communities by the alien succulent *Carpobrotus edulis* Ecology 74: 83–95

D'Antonio CM, Vitousek PM (1992) Biological invasions by non-indigenous grasses, the grass/fire cycle, and global change Ann Rev Ecol Syst 23: 63–87

Deleuil G, transmise par Emberger L (1950) Explication de la présence de certains thérophytes rencontrés parfois dans les associations du *Rosmarino-Ericion*. Académie des sciences séance du 25 juin 1951. 2477.

Ewel JJ, O'Dowd DJ, Bergelson J, Daehler CC, D'Antonio CM, Gomez D, Gordon DR, Hobbs RJ, Holt A, Hopper KR, Hughes CE, Lahart M, Leakey RRB, Lee WG, Loope LL, Lorence DH, Louda SM, Lugo AE, Mcevoy PB, Richardson DM, Vitousek PM (1999) Deliberate introductions of species: research needs? Benefits can be reaped, but risks are high. BioScience 49: 619–630

Harrington GN (1991) Effect of soil moisture on shrub seedling establishment and survival in a semi-arid grassland. Ecology 72: 1138–1149

Heisey RM (1990) Allelopathic and herbicidal effects of extracts from tree of heaven (*Ailanthus altissima*). Am J Bot 77(5): 662–670

Hierro JL, Callaway RM (2003) Allelopathy and exotic plant invasion. Plant Soil 256: 29–39

Hobbs RJ, Huenneke LF (1992) Disturbance, Diversity, and Invasion: Implications for Conservation. Conserv Biol 6: 324–337

Huenneke LF, Hamburg SP, Koide R, Mooney HA, Vitousek PM (1990) Effects of soil resources on plant invasion and community structure in California serpentine grassland. Ecology 71: 478–491

Hulst RB, Shiply B, Theriault A (1987) Why is *Rhinanthus minor* (Scrophulariaceae) such a good invader? Can. J Bot 65: 2373–2379

Lambinon J (1997) Introduction of non-native plants into the natural environment. Nature and environment [Council of Europe]. 87: 29p

Laterra P, Bazzalo ME (1999) Seed-to-seed allelopathic effects between two invaders of burned Pampa grasslands. Weed Research 39: 297–308

Lawrence JG, Colwell A, Sexton OJ (1991) The ecological impact of allelopathy in *Ailanthus altissima* (simaroubaceae). Am J Bot 78(7): 948–958

Mack MC, D'Antonio CM (1998) Impacts of biological invasions on disturbance regimes. Trends Ecol Evol 13: 195–198

Médail F, Quézel P (1997) Hot-spots analysis for conservation of plant biodiversity in the Mediterranean Basin. Mediterranean Plant Biodiversity 84(1): 112–127

Médail F, Verlaque R (1997) Ecological characteristics and rarity of endemic plants from S-E. France and Corsica. Implications for biodiversity conservation. Biol Conserv 80: 269–281
Miller DA (1996) Allelopathy in forage crop systems Agronomy Journal 88: 854–859
Molina A, Reigosa MJ, Carballeira A (1991) Release of allelochemical agents from litter, throughfall, and topsoil in plantations of *Eucalyptus globulus* Labill in Spain. J Chem Ecol 17(1): 147–160
Muller CH (1969) Allelopathy as a factor in ecological process Vegetatio 18: 48–357
Nilsson MC (1994) Separation of allelopathy and resource competition by the boreal dwarf shrub *Empetrum hermaphroditum*. Oecologia 98: 1–7
Noble IR (1989) Attributes of invaders and the invading process: terrestrial and vascular plants. Pp 301–313 *in* Drake JA, Mooney HA, Di Castri F, Groves RH, Kruger FJ, Rejmanek M, Williamson M (eds). Scope 37: Biological Invasions: A Global Perspective. USA- New York: John Wiley and Sons
Oleszek WA, Hoagland RE, Zablotowicz RM (1999) Ecological significance of plant saponins Principles and Practices in Plant Ecology: 451–465
Orions GH (1986) Site characteristics favoring invasions. Pp 133–148 i*n*: Drake JA, Mooney HA, Di Castri F, Groves RH, Kruger FJ, Rejmanek M, Williamson M (eds). Scope 37: Biological Invasions: A Global Perspective. USA-New York: John Wiley and Sons
Quayyum HA, Mallik AU, Orr DE, Lee PF (1999) Allelopathic potential of aquatic plants associated with wild rice: II. Isolation and identification of allelochemicals. J Chem Ecol 25(1): 221–228
Reichard SH (1997) Predicting invasions of woody plants introduced into North America. Conserv Biol 11: 193–202
Reigosa MJ, Gonzalez L, Souto XC, Pastoriza JE, (2000) Allelopathy in forest ecosystems. Pp 183–193 *in* Tauro P (eds) Allelopathy in Ecological Agriculture and Forestry. Netherlands: Kluwer Academic Publishers
Rejmanek M (1996) A theory of seed plant invasiveness: the first sketch. Biol Conserv 78: 171–181
Rice EL (1984) Allelopathy. 2nd Ed GB-London: Academic press
Ridenour W, Callaway RM (2001) The relative importance of allelopathy in interference: the effects of an invasive weed on a native bunchgrass Oecologia 126(3): 444–450
Rutherford MC, Powrie LW (1993) Allelochemic control of biomass allocation in interacting shrub species. Journal of Chemical Ecology 19(5): 893–906
Scherrer B (1984) Biostatistique. Chicoutimi: Gaëtan Morin publ
Torck M, Pinkas M (1980) Sur quelques polyphénols de *Medicago arborea* L. (Légumineuses). Plantes Médicinales et Phytothérapie 1(1): 20–25
Vaughn SF, Berhow MA (1999) Allelochemicals isolated from tissues of the invasive weed garlic mustard (*Alliaria petiolata).* J Chem Ecol 25: 2495–2504
Vila M, D'Antonio CM (1998). Fruit choice and seed dispersal of invasive vs. non-invasive *Carpobrotus* (Aizoaceae) in coastal California. Ecology 79: 1053–1060
Vivanco J, Bias H, Stermitz F, Thelen G, Callaway R (2004) Biogeographical variation in community response to root allelochemistry: novel weapons and exotic invasion. Ecology Letters 7: 285–292
Vivrette NJ, Muller CH (1977) Mechanism of invasion and dominance of coastalgrassland by *Mesembyranthemum crystallinum.* Ecol Monogr 47: 301–318
Vyvyan JR (2002) Allelochemicals as leads for new herbicides and agrochemicals. Tetrahedron 58: 1631–1649
Williamson GB (1990) Allelopathy, Koch's postulates, and the neck riddle. Pp 143–162 *in* Grace JB, Tilman T (eds). Perspective on Plant Competition. USA-San Diego, CA: Academic Press, Inc
Williamson M (1996) Biological Invasions. GB-London: Chapman and Hall
Williamson M (1999) Invasions. Ecography 22: 5–12
Wiser SK, Allen RB, Clinton PW, Platt KH (1998) Community structure and forest invasion by an exotic herb over 23 years. Ecology 79: 2071–2081
Xuan TD, Shinkichi T, Khanh TD, Min CI (2005) Biological control of weeds and plant pathogens in paddy rice by exploiting plant allelopathy: an overview Crop Protection 24: 197–206
Zedler PH, Scheid GA (1988) Invasion of *Carpobrotus edulis* and *Salix lasiolepis* after fire in a coastal chaparral site in Santa Barbara County, California. Madrono 35: 196–201