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REVIEW ARTICLE

Benzoxazinoids in Rye Allelopathy - From Discovery to Application in Sustainable Weed Control and Organic Farming

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Abstract The allelopathic potency of rye (*Secale cereale* L.) is due mainly to the presence of phytotoxic benzoxazinones—compounds whose biosynthesis is developmentally regulated, with the highest accumulation in young tissue and a dependency on cultivar and environmental influences. Benzoxazinones can be released from residues of greenhouse-grown rye at levels between 12 and 20 kg/ha, with lower amounts exuded by living plants. In soil, benzoxazinones are subject to a cascade of transformation reactions, and levels in the range 0.5–5 kg/ha have been reported. Starting with the accumulation of less toxic benzoxazolinones, the transformation reactions in soil primarily lead to the production of phenoxazinones, acetamides, and malonamic acids. These reactions are associated with microbial activity in the soil. In addition to benzoxazinones, benzoxazolin-2(3*H*)-one (BOA) has been investigated for phytotoxic effects in weeds and crops. Exposure to BOA affects transcriptome, proteome, and metabolome patterns of the seedlings, inhibits germination and growth, and can induce death of sensitive species. Differences in the sensitivity of cultivars and ecotypes are due to different species-dependent strategies that have evolved to cope with BOA. These strategies include the rapid activation of detoxification reactions and extrusion of detoxified compounds. In contrast to sensitive ecotypes, tolerant ecotypes are less affected by exposure to BOA. Like the original compounds BOA and MBOA, all exuded detoxification products are converted to phenoxazinones, which can be degraded by several specialized fungi via the Fenton reaction. Because of their selectivity, specific activity, and presumably limited persistence in the soil, benzoxazinoids or rye residues are suitable means for weed control. In fact, rye is one of the best cool season cover crops and widely used because of its excellent weed suppressive potential. Breeding of benzoxazinoid resistant crops and of rye with high benzoxazinoid contents, as well as a better understanding of the soil persistence of phenoxazinones, of the weed resistance against benzoxazinoids, and of how allelopathic interactions are influenced by cultural practices, would provide the means to include allelopathic rye varieties in organic cropping systems for weed control.

Keywords Rye (*Secale cereale* L.) allelopathy · Benzoxazinoids · Phenoxazinones · Detoxification · Biodegradable allelochemicals · Organic farming · Soil persistence · Sustainable weed control

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Introduction

Secale cereale L. grew first as a weed in wheat and barley fields until it became a crop on its own about 2,000 years ago. Although the direct genetic origin is uncertain, *Secale montanum* (native to Southern Europe) or *S. anatolicum* (native to Anatolia) are believed to be ancestors of *Secale cereale* (Chikmawati et al., 2005). Today, rye is used as a grain, forage, green manure crop or for hay, as a cover crop to reduce soil erosion and for retention of soil nitrates

mainly in Europe, North America, and Northern Asia. The plant can grow on poor and acid soils, has a higher frost and drought resistance than wheat, and is therefore particularly suitable for cropping in mountain or infertile areas (Clark, 2007). During the last decades, the use of rye as a cover crop or mulch for allelopathic weed control, e.g., in maize, cotton, and soybean fields, has gained importance (Barnes and Putman, 1983; de Bruin et al., 2005). In this review, we focus on the main secondary compounds that function as allelochemicals/bioherbicides, the fate of these molecules in the environment, as it is known so far, and the ability of rye to reduce weed growth in organic farming. We also focus on the resistance response of some weeds to these compounds, since this ability may limit the use of rye residues as a suitable tool of weed control.

Major Secondary Compounds and Allelochemicals

Major secondary products of *Secale cereale* are phenylpropanoids, particularly ferulic acids, such as 2-(*E*)-0-feruloylgluconic acid in primary leaves or ferulic acid dehydrodimers, *p*-coumaric and sinapic acids in kernels (Strack et al., 1986; Wojcik-Wojtkowiak et al., 1990; Andreasen et al., 2000). Several flavonoids are present in young rye leaves. In addition to cyanidine glycosides, isovitexine 2″-*O*-glycosides, apigenine-glycosides, and luteoline-0-glucuronides are found in primary leaves (Strack et al., 1982; Dellamonica et al., 1983; Schulz et al., 1985; Schulz and Weissenböck, 1987, 1988). Whereas *p*-coumaric acid, ferulic acid, and two isovitexine-glycosides accumulate in the adaxial and abaxial epidermis, the mesophyll contains the two luteoline-glucuronides. The most important secondary products are glucosylated benzoxazinones (BX), 2,4-dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIBOA glucoside) in the shoots and 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIMBOA glucoside) in the roots (Copaja et al., 2006). They occur together with the aglucones and their degradation products, the benzoxazolinones BOA and MBOA (BXL), (Table 1). The discovery of natural benzoxazinones started from the observation that rye plants exhibited a higher resistance against pathogenic fungi. Thus, the first identified plant benzoxazinoids were from rye (Virtanen and Hietala, 1960; Hofman and Hofmanova, 1969; Barnes et al., 1987; Hartenstein and Sicker, 1994; Sicker and Schulz, 2002; Finney et al., 2005; Niemeyer, 2009). The aglycones and their derivatives are mainly responsible for the phytotoxic effects of rye residues, but they may act in concert with other compounds, such as ferulic acid and related compounds, luteoline glucuronides, β-phenyllactic acid, and β-hydroxybutyric acid (Shilling et al., 1985). Allelopathic effects of luteoline glucuronides and ferulic acid have been described by Booker et al. (1992) and Beninger and Hall (2005), but these compounds are less toxic than DIBOA and BOA. Tissues of young rye plants have the highest concentration of simple phenolic allelochemicals. However, from results of bioassays, it was concluded that these phenolics cannot be the major compounds responsible for growth inhibition. A strong participation of BOA and DIBOA was assumed (Wojcik-Wojtkowiak et al., 1990). The studies of Barnes and Putnam (1983, 1986), Shilling et al. (1985), Barnes et al. (1987), Burgos et al. (1999), and the groups of N.M. Niemeyer and L. A. Weston also verified DIBOA and the much more stable BOA as the major phytotoxic compounds in rye (Queirolo et al., 1981; Niemeyer et al., 1982; Niemeyer, 1988, 2009; Mwaja et al., 1995; Weston, 1996; Weston and Duke, 2003).

Table 1 Compounds/allelochemicals found in young rye plants

Compound	Reference
p-Hydroxybenzoic acid	Wojcik-Wojtkowiak et al., 1990
Protocatechuic acid	Wojcik-Wojtkowiak et al., 1990
Gallic acid	Wojcik-Wojtkowiak et al., 1990
Vanillic acid	Wojcik-Wojtkowiak et al., 1990
Syringic acid	Wojcik-Wojtkowiak et al., 1990
p-Coumaric acid	Wojcik-Wojtkowiak et al., 1990
Ferulic acid/conjugates	Strack et al., 1986
Cyanidin glycosides	Strack et al., 1982
Apigenin-glycosides	Strack et al., 1982
Isovitexin glucosides	Dellamonica et al., 1983
Luteolin glucuronides	Schulz et al., 1985; Schulz and Weissenböck, 1987; 1988
DIBOA (glucoside)	Barnes et al., 1987; Hietala and Virtanen, 1960; Hartenstein and Sicker, 1994
DIMBOA (glucoside)	Hofman and Hofmanova, 1969; Copaja et al., 2006
Benzoxazolinones BOA	Barnes et al., 1987
β-Phenyllactic acid	Shilling et al., 1985
β-Hydroxybutric acid	Shilling et al., 1985

BX-Genes and Biosynthesis

Benzoxazinoids are characteristic secondary compounds not only of rye but also of several other species of the Poaceae, such as maize, triticale, and wheat, and of some dicot species belonging to the Acanthaceae, Scrophulariaceae, and Lamiaceae. Within the Ranuculaceae, only *Consolida orientalis* contains these compounds (Sicker et al., 2000; Sicker and Schulz, 2002; Schullehner et al., 2008; Frey et al., 2009; Bertholdsson et al., 2012). Benzoxazinoids do not occur in Sorghum or rice, which produce other highly active allelochemicals (see reviews of Kato-Noguchi and Peters, 2013; Weston et al., 2013). The biosynthesis has been investigated in maize (Frey et al., 1997, 2003, 2009; Glawischnig

et al., 1999; von Rad et al., 2001; Jonczyk et al., 2008; Schullehner et al., 2008; Dick et al., 2012). Following the synthesis of indole by BENZOXAZINELESS1 BX1, four cytochrome P450 monooxygenases (BX2-BX5) complete the formation of the benzoxazinone (DIBOA) molecule. DIBOA is subsequently glucosylated at the 2-position by specific glucosyltransferase(s), (Dick et al., 2012). Two homologous glucosyltransferase genes, *Bx8* and *Bx9*, have been identified, and the resulting enzymes are both able to glucosylate DIBOA. However, BX9 seems to be more important for detoxification than for biosynthesis. The preferred involvement in detoxification might originate from adaptation during evolution. (von Rad et al., 2001; Schulz et al., 2011,. unpublished). The product DIBOA-glucoside is the precursor of DIMBOA-glucoside. The final steps of DIMBOA-glc synthesis are catalyzed by dioxygenase BX6 and methyltransferase BX7. Whereas BX1 is located in the plastids, BX2, BX3, BX4, and BX5 are associated with microsomes, BX8, BX9, BX6, and BX7 are cytosolic enzymes. The glucosylated end products are stored in the vacuoles of cells in young tissues of roots and leaves. In rye, the subcelluar localization of the enzymes has not been investigated but is probably similar to that of maize.

In maize, *BX8* is located in a cluster with the benzoxazinone biosynthetic genes *Bx1- Bx5* on chromosome 4, whereas *BX9* is located at the short arm of chromosome 1. In rye, the *ScBX1-BX5* genes are dispersed to chromosomes 7R and 5R (Nomura et al., 2003), but the glucosyltransferase gene *ScGT* is located on chromosome 4R (Sue et al., 2011). Sue et al. (2011) suggested that the biosynthesis is not necessarily dependent on gene clustering. A similar situation was found with *Triticum aestivum*. The specific plastid-localized β-glucosidases were first isolated from maize (Cicek et al., 2000). The β-glucosidase releases the bioactive aglucon DIBOA after tissue damage and destruction of the cell compartments. A corresponding rye gene is located at chromosome R2 (Sue et al., 2011). Phylogenomics of the benzoxazinone biosynthesis in Poaceae is not further described here, as it is beyond the scope of this review. Moreover, two excellent articles which cover this interesting topic were recently published by Dick et al. (2012) and Dutartre et al. (2012).

BX-Gene Activities

All Sc*BX* genes involved in the biosynthesis exhibit a high expression during the seedling stage, whereas transcript levels decline with further development (La Hovary, 2011; Sue et al., 2011). This gene expression pattern is similar to that found in maize (Frey et al., 1997, 2009; Ebisui et al., 2001; Nomura et al., 2005) and matches the benzoxazinoid accumulation. La Hovary (2011) found a significant increase in BX content after wounding in immature, but not in mature rye leaves. This was associated with a 3.5 fold increase in *ScBX1* and *ScBX2* transcription. Methyl jasmonate treatment also increased BX content in immature leaves, but there was no corresponding increase in *ScBX1* and *ScBX2* transcripts.

An up-regulation of BX biosynthesis by pathogens and herbivore attack has been reported in maize (Ahmad et al., 2011; Glauser et al., 2011). Here, the increase in BX accumulation was attributed to a highly increased expression of *Igl* (indol-3-glycerolphosphate lyase gene), and of BX1, although to a much lesser extent. Whereas the expression of *BX1* is developmentally regulated, *Igl* expression is induced by herbivore feeding, wounding, or methyl jasmonate treatment, and the Igl reaction results in the release of the volatile indole (Frey et al., 2000, 2004). Currently, it is unknown whether rye possesses a stress inducible IGL enzyme that could contribute to BX biosynthesis.

Benzoxazinoid Content and Future Breeding Aims

There is increasing interest in breeding rye cultivars with high allelopathic potential. Brooks et al. (2012) investigated DIBOA contents in a synthetic population of half-sib families at the flag leaf stage. They identified low and high producers among the different genotypes. They differed in the BOA and DIBOA content (from 0.52 to 1.15 mg/g dry tissue), and those with the highest content of benzoxazinoids were also most suppressive on weeds. Another indication of heritability was observed by Burgos et al. (1999).

Rye cultivars have been thoroughly investigated for their BX contents during plant development. In Table 2, the BX concentrations determined in shoot tissue of 17 rye cultivars are presented. Depending on the cultivar and developmental stage, total BX contents range from 161 to 1,981 $\mu g\,g^{-1}$ d.m. The highest amounts were detected in 111 day old plants of the cultivars Aroostock, Bonel, Wheeler, and Wrens Abruzi. All these cultivars showed a 62–63 % reduction of the total BX content at later growth stages (136 up to 240 days), (Burgos et al., 1999; Weston and Duke, 2003; Reberg-Horton et al., 2005; Rice et al., 2005; La Hovary, 2011; Sue et al., 2011). As mentioned above, BX synthesis is generally highest in young tissue and decreases during plant development. Since the shoot and root biomasses of the plant increase during the entire vegetative growth phase, the absolute BX content is highest in the youngest tissues, and at the end of the growth phase, when biomass production is maximal (La Hovary, 2011).

A number of studies have shown that the variable capacity to suppress weeds can be explained in part by the ability of the plants to secrete allelochemicals (Weih

Table 2 Age and Cultivar Dependent Bx Concentration In Shoot Tissue (selection)

Rye cultivar	Sowing-harvest (days)	DIBOA content µg/g^{-1}dm	BOA content µg/g^{-1}dm	Total BX µg/g^{-1}dm	Reference
Abruzzi	56	287	114	407	Rice et al., 2005
Abruzzi	190	108	96	208	Rice et al., 2005
Aroostook	111			1981	La Hovary, thesis 2011
Aroostook	136			542	La Hovary, thesis 2011
Bonel	104	~1800			Reberg-Horton et al., 2005
Bonel	161	below 200			Reberg-Horton et al., 2005
Bonel	111			1960	La Hovary, thesis 2011
Bonel	136			554	La Hovary, thesis 2011
Born	121	177	n.d.	177	Tabaglio et al., 2008
Emerald	20	2.1 mmol/kg fr.w.			Argandoña et al., 1980
Fasto	106	534	11	545	Tabaglio et al., 2008
Forestier	126	338	62	400	Tabaglio et al., 2008
Forrajero Baer	seedlings	1300 (fr.w.)			Peréz and Ormeño-Nuñez, 1991
Matador	121	329	n.d.	329	Tabaglio et al., 2008
NC unnamed	104	~1500			Reberg-Horton et al., 2005
NC unnamed	161	~0			Reberg-Horton et al., 2005
Nikita	121	286	1	287	Tabaglio et al., 2008
Primizia	107	283	114	397	Tabaglio et al., 2008
Protector	106	225	n.d.	225	Tabaglio et al., 2008
Tetra Baer	seedlings	800 (fr.wt.)			Peréz and Ormeño-Nuñez, 1991
Trevisio	121	266	n.d.	266	Tabaglio et al., 2008
Wheeler*	75	532.8	16.8	550	Mwaja et al., 1995
Wheeler	240	138	23.5	161	Mwaja et al., 1995
Wheeler	111			1922	La Hovary, thesis 2011
Wheeler	136			521	La Hovary, thesis 2011
Wheeler	104	~1600			Reberg-Horton et al., 2005
Wheeler	161	below 100			Reberg-Horton et al., 2005
Wrens Abruzzi	111			1744	La Hovary, thesis 2011
Wrens Abruzzi	136			472	La Hovary, thesis 2011
Wrens Abruzzi	104	~1000			Reberg-Horton et al., 2005
Wrens Abruzzi	161	~0			Reberg-Horton et al., 2005

et al., 2008). These results may indicate a potential of allelopathic traits for use as selection criteria in breeding programs of cereals (Bertholdsson, 2010). Genetic and molecular biological work on rye allelopathy or allelochemicals includes initial studies on gene functions and the identification of Quantitative Traits Loci (QTLs) (Niemeyer and Jerez, 1997; Wu et al., 2003; Macías et al., 2007; Dick et al., 2012). In cereals, little genetic information is available on quantitative differences in allelochemical production of cultivars with different allelopathic activity (Belz, 2007). The identification of QTLs for allelopathic functions represents a strategy to enhance allelopathic activity in crops by using marker-assisted selection.

Influence of Stress and Environmental Factors on BX Contents

Aside from the genotype, the concentrations of BOA and DIBOA depend on plant organ, plant age, on the fertilization regime, and on environmental factors: temperature, water supply, photoperiod, UV irradiation, and light intensity, which have been reviewed thoroughly by Niemeyer (2009).

Mwaja et al. (1995) grew Wheeler rye for 75 days at low, medium, and high amounts of N, P, and K, whereas micronutrients, S, Ca, and Mg were kept constant. Analyses of the DIBOA and BOA contents in the dried shoot material revealed a higher benzoxazinoid production under low and

medium fertilization regimes. Severe nitrogen deficiency, however, reduced the benzoxazinoid content. In comparison to 0 N regimes, application of 50 kg N ha^{-1} led to an increase of benzoxazinoid content by 41 % (Gavazzi et al., 2010). Stress conditions do not only enhance benzoxazinoid content, but can induce shifts in the organ specific production of the compounds (Collantes et al., 1999; Gianoli et al., 1999). Stress due to defoliation led to an allocation of BX from shoots to roots and root exudates. The molecular signals necessary to initiate long distance transport of benzoxazinoids are not known at present.

Release of Benzoxazinoids

Benzoxazinoids are released passively from plant residues or actively by root exudation. Peréz and Ormeño-Nuñez (1991) determined the DIBOA contents in root exudates of two rye varieties. Tetra Baer released only 70 nmol DIBOA kg^{-1} fresh weight, but the cultivar Forrajero Baer released 25 μmol DIBOA kg^{-1} fresh weight. DIMBOA was detected in neither case. It is not known if the two cultivars do not synthesize DIMBOA in the roots or if this compound is not exuded. In maize, the mode of molecular transportation of BXs into the apoplast of roots is presently under investigation (Ahmad et al., 2011).

Evidence for cultivar-dependent differences in the composition and amount of BX in root exudates is emerging. This aspect warrants further attention, not only because the allelopathic activity of living rye plants is modified but also because the interaction of the root and micro-organisms may be affected by the BX concentration in the rhizosphere, as described for maize and wheat (Saunders and Kohn, 2008; Chen et al., 2010; Neal et al., 2012). The amounts of BX released by rye residues are estimated to be between 12 and about 20 kg/ha, depending on the date of plant death and the cultivar (Argandoña et al., 1980; Barnes and Putnam, 1987; Mwaja et al., 1995). The afore mentioned BX values were estimated from greenhouse-grown rye, although the levels derived from field-grown rye have been reported to be between 0.5 and 5 kg ha^{-1} (Barnes and Putnam, 1987; Reberg-Horton et al., 2005).

Chemical Stability of Benzoxazinoids

The fate of the secondary metabolites after release/excretion into the soil has recently become the focus of research (Macías et al., 2006a; Jilani et al., 2008; Kong et al., 2008; Tharayil, 2009; Zhang et al., 2010; Ehlers, 2011; Chen et al., 2011; Cipollini et al., 2012). The interest in this research area stems from the finding that the cyclic hydroxamic acids with a (2*H*)-1,4-benzoxazin-3 (4*H*)-one skeleton are chemically labile, and are prone to degradation to benzoxazolinones. Furthermore, the transformation compounds, such as 2-aminophenoxazin-3-one, sometimes show a higher bioactivity than the parent compounds (Gealy et al., 1996; Coja et al., 2006; Bacon et al., 2007; Kato-Noguchi et al., 2010). Thus, any study of the role of benzoxazinones in allelopathic interactions should take into account the contribution of degradation compounds to overall bioactivity.

Interestingly, both DIBOA and DIMBOA have been found to be unstable in aqueous solutions (Bredenberg et al., 1962; Woodward et al., 1978; Bravo and Niemeyer, 1985). Temperature and pH are two factors that determine the kinetics of the degradation, with the influence of pH being crucial. Thus, the half-life for chemical degradation of DIBOA at 75 °C increases from 16 hr at pH 4 to 4.5 min at pH 8 (Bredenberg et al., 1962). Similarly, the half life of DIMBOA is 7 hr at 25 °C and pH 7.5, and about 20 hr at pH 6 (Woodward et al., 1978). This strong influence of pH is related to deprotonation of the hydroxamic acid, a process that is necessary to initiate the degradation (Bravo and Niemeyer, 1985). Two different mechanisms have been proposed for the transformation reactions (Fig. 1), although it is difficult to postulate a completely satisfactory mechanism. Studies carried out on soil-water suspension have revealed similar results, with half-life values of 1 day and 30 min for DIBOA and DIMBOA, respectively (Macías et al., 2004, 2005a). Based on these findings, the majority of studies on activity and degradation of these allelochemicals have been conducted on their immediate degradation products, the benzoxazolinones. In juice from injured maize cells, the half life of DIMBOA is about 1 day (Woodward et al., 1978), which demonstrates the biological relevance of the degradation.

Microbial Activities and BX Transformation Products

BOA and MBOA are much more stable than DIBOA and DIMBOA, and rapid degradation requires microbial activity. As an example, when MBOA was placed in a previously sterilized soil, the concentration did not change over a period of 4 days (Macías et al., 2004). Identical results were obtained when BOA was incubated in media with *Plectosporium tabacinum* and *Glioclaudium cibotii* for 24 days (Zikmundova et al., 2002a), indicating that some microorganisms require priming or are unable to start the degradation process.

The degradation of BOA and MBOA has been extensively studied. The first process seems to be the conversion to the corresponding aminophenol (Niemeyer, 1988). The role of 2-aminophenol as a key intermediate in the degradation of BOA and the lactam HBOA has been

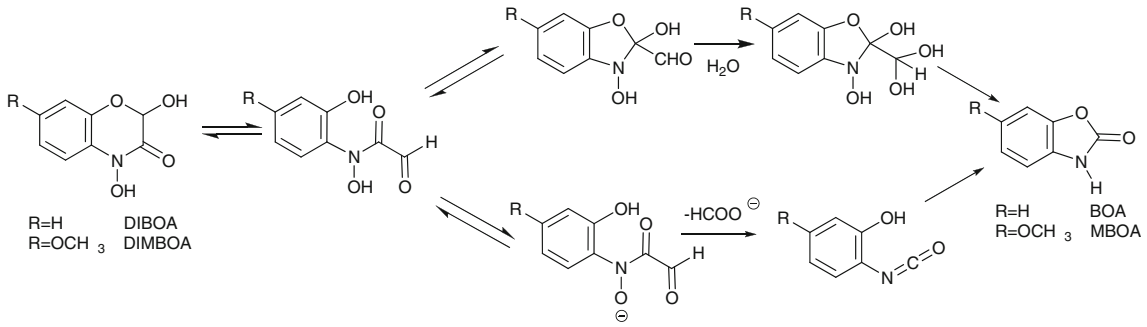


Fig. 1 The two possible mechanisms for the transformation of DIBOA/DIMBOA to BOA/MBOA

confirmed through isotopic labeling experiments with metabolic intermediates (Zikmundova et al., 2002a). Aminophenols can be transformed mainly into three different classes of compounds: aminophenoxazinones, acetamides, and malonamic acids, (Fig. 2). These compounds were identified when experiments were conducted with endophytic fungi (Zikmundova et al., 2002a, b), and they are produced by different species of fungi and bacteria (Friebe et al., 1996, 1998; Yue et al., 1998). Subsequent *N*-oxidation that led to 2-(*N*-hydroxy)acetylamino-phenoxazin-3-one (NHAAPO) and finally to 2-(2-hydroxyacetyl)amino-3*H*-phenoxazin-3-one (HAAPO) was also described (Zikmundova et al., 2002a). It is worth noting that the dimerization of aminophenols that gives rise to the corresponding aminophenoxazin-3-one proceeds without the intervention of microorganisms. Thus, oxidation of aminophenol, mediated by oxygen in the air, produces aminophenoxazinone (APO), which was first reported as 2,2′-oxo-1,1′-azonezene (AZOB) (Nair et al., 1990) and corrected by Gagliardo and Chilton in 1992 to 2-amino-3H-phenoxazin-3-one. Oxidation of aminophenol is a commonly used procedure for the preparation of APO in the laboratory (Macías et al., 2006b).

Fate of the Compounds in the Soil

During degradation in the soil, different metabolites accumulate in a concentration and soil type dependent manner. The differences can be related to the density and diversity of microorganisms associated with the plant and those in the soil (Hashimoto and Shudo, 1996; Grayston et al., 1998; Glenn et al., 2001). Indeed, compounds released from plants depend on the plant species, and can also influence the micro flora associated with the root system. The microorganisms may have different capacities to metabolize these compounds. Another important aspect is the humidity of the sample. Gagliardo and Chilton (1992) did not find any transformation after the incubation of BOA in a sandy loam soil, whereas the transformation was complete in 4 days in liquid media.

Regarding the concentration, the biotransformation of 3 nmol BOA g^{-1} in soil was almost complete in the first 24 hr, and neither APO nor acetylaminophenoxazinone (AAPO) were detected (Gents et al., 2005; Understrup et al., 2005). The process was retarded at higher concentrations in the same soil. At 3,000 nmol g^{-1} soil, the biotransformation was not complete after 30 days, and the maximum level

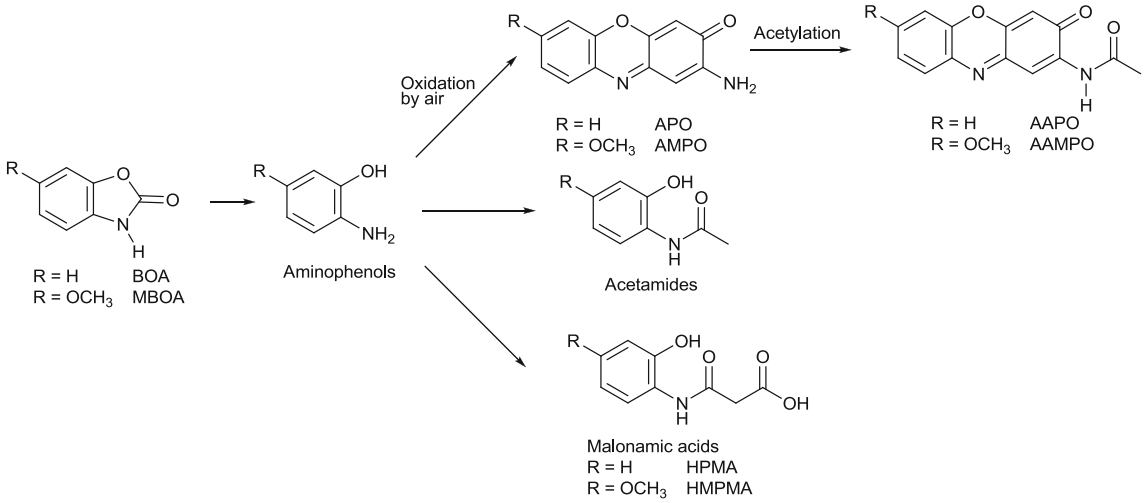


Fig. 2 Transformation of aminophenols into three different classes of compounds: Aminophenoxazinones, acetamides and malonamic acids

of APO was reached after 5 days, after which it decreased with the appearance of AAPO. At 30,000 nmol g^{-1}, soil degradation was even slower, with BOA detected even after 90 days and a maximum concentration of APO achieved after 50 days.

The biodegradation of DIBOA-Glc, DIBOA, and BOA also was carried out in a soil in which wheat varieties had previously been cultivated (Macías et al., 2005a). Concentrations between 0.25 and 5 mg were used. The complete set of compounds from the pathway of DIBOA-Glc to APO was observed, but AAPO could not be detected. Conversion of BOA was close to 99 % after 10 days at 2 mg g^{-1} soil, and this decreased with concentration. Under these conditions, the APO concentration persists without significant variation after 90 days. This suggests that during the degradation process the concentrations of APO become toxic to the associated microorganisms, thus preventing subsequent biotransformation. APO is toxic to a number of organisms, including bacteria and fungi. Similar results were obtained when the same amounts of DIMBOA and MBOA were subjected to degradation in the same soils (Macías et al., 2004). A significant influence between dose and degradation was observed, with a maximum conversion rate at the lowest concentration. Once again, transformation of the aminophenoxazinone AAPO was not detected after 90 days.

The incorporation of wheat and rye shoots into the soil is a common practice in crop rotation (see below for more details). The study of Krogh et al. (2006) provided information about the dynamic pattern of biologically active benzoxazinone derivatives in soil after the incorporation of wheat and rye shoots. The highest concentrations of most of the compounds were measured at day 1 after incorporation. A maximum concentration was reached at day 4 for a few of the compounds.

In the wheat experiments, MBOA, HMBOA, and HBOA were detected, but phenoxazinones were not observed. The degradation pattern for the rye system was more complex. In the first 2 days of incubation, MBOA and 2,4-DIBOA were detected as the main allelochemicals, along with HBOA, HMBOA, and BOA in decreasing order. Later in the incubation period, some APO was detected, and the amount of HBOA increased considerably before decreasing once again. The profiling of the benzoxazinone metabolites and their derivatives in soil was dynamic and time-dependent.

Recently, Rice et al. (2012) reported the concentrations of benzoxazinoids in field soils treated with rye cover crop and some unexpected results were obtained. First, a different proportion of methoxylated and non-methoxylated derivatives were found in the rye residue compared to soil that contained predominantly methoxy compounds. This was explained by the presence of residual roots that remain in the soil after harvest. Even more surprising was the finding that the relative amounts of the different compounds did not change during the two weeks in which benzoxazinoids persisted in soil. Almost no degradation was observed, and concentrations of APO and AMPO were negligible. Despite the concentrations of benzoxazinoids being seemingly too low to cause inhibition, phytotoxic effects were found in these soils. Another unexpected finding was the minimal movement of BOA and MBOA into the soil column, with more than 97 % MBOA remaining in the top 1-cm of the soil profiles.

Structure-Activity Relationship

A complete structure-activity relationship study (SAR) of the phytotoxic effects of these compounds (shown in Figs. 1, 2, and 3) has been performed. Targets were cultivars of the species *Lepidium sativum*, *Lactuca sativa*, *Solanum lycopersicon* (formerly *Lycopersicon esculentum)*, *Triticum aestivum*, *Allium cepa*, *Lolium rigidum*, *Avena fatua*, and *Echinochloa crus-galli* (Macías et al., 2005a, b; 2006a, b). The natural allelochemicals DIBOA and DIMBOA, and their synthetic analogs D-DIBOA, D-DIMBOA, and ABOA, were the most active compounds. The other analyzed chemicals, which are intermediates in natural benzoxazinone degradation pathways, had moderate or null activity, except for APO, which showed high inhibitory effects. The most affected parameter was root length for all the active compounds, followed by shoot length. Regarding selectivity, wheat and lettuce were the least affected species. These selective characteristics, which were particularly observed for DIMBOA, D-DIMBOA, DIBOA, and D-DIBOA, indicated that these natural allelochemicals are not involved in the intraspecific competition phenomena observed for wheat. These compounds and APO inhibited wheat growth. However, the concentrations used in these assays here were significantly higher than the natural ones.

Cluster analysis of all data showed that the most active chemicals were the aminophenoxazine APO and the modified benzoxazinone ABOA (Fig. 4). The synthetic benzoxazinones D-DIBOA, D-DIMBOA, the natural products DIMBOA and DIBOA, and the degradation product 2-aminophenol showed significant activities. Lower activities were found in this system for the degradation products (AAPO, BOA, MBOA, AMPO, and AAMPO) and the lactams D-HMBOA and D-HBOA. Natural lactams (HBOA and HMBOA) and malonamic acids (HPMA and HMPMA) were not included in these analyses due to their lack of phytotoxic effect. Thus, the transformation from benzoxazinone to the benzoxazolinone skeleton leads to dramatic reductions in phytotoxicity.

Studies on the structural requirements for phytotoxicity on the benzoxazinone skeleton revealed that the oxygen atom at N-4 is crucial for the phytotoxic effect, since all 4-

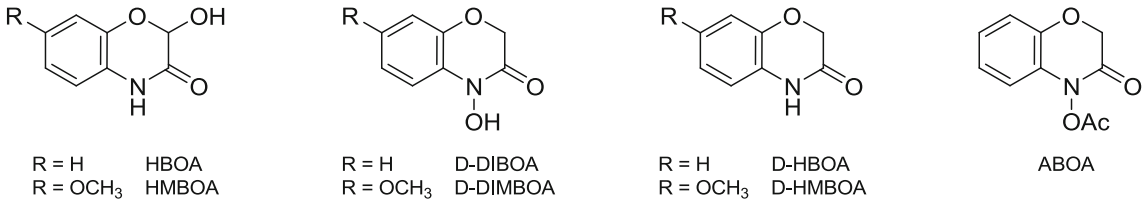


Fig. 3 Structures of the natural BX allelochemicals and their synthetic analogs

hydroxy benzoxazinones (DIBOA, DIMBOA, D-DIBOA, and D-DIMBOA) are more active than the corresponding lactams (HBOA, HMBOA, D-HBOA, and D-HMBOA).

A similar SAR study was performed with respect to the growth inhibition and α-amylase activity in cress seedlings (Kato-Noguchi et al., 2010). Benzoxazinones and their degradation products inhibited root growth and α-amylase activity. The structure-activity relationship for these compounds suggests that systems that have a benzoxazinone skeleton are most active. Furthermore, the presence of a hydroxyl group at position C-2 does not affect inhibitory activity on root growth and α-amylase activity, whereas a hydroxyl group at position N-4 on the skeleton is essential for inhibitory activity. Additionally, a positive correlation was found between the concentration-response curves and I_{50} values for root growth and those of α-amylase activity. Therefore, a reduction in α-amylase activity after exposure to these compounds might contribute to root growth inhibition of cress seedlings.

In general, the contribution of degradation products to bioactivities must be taken into account in any biological study of benzoxazinoids, since half-life times are, in some cases, as short as the bioassay time (Fritz and Braun, 2006; Idinger et al., 2006; Macías et al., 2009).

Efffects of Benzoxazinoids in Target Plants and Weed Suppression

The mutagenic, electrophilic benzoxazinoids interact with proteins, intercalate with nucleic acids (Dufall and Solomon, 2011), and are deleterious for many cellular structures and activities. They influence plant cells on the transcriptome, proteome, and metabolome level. Often, experiments have been performed with BOA and not with DIBOA because of the relatively fast transformation of DIBOA to the more stable BOA in non-sterile systems. BOA is actively absorbed by all tested plants, but the exact mechanism of uptake is not understood (Wieland et al., 1998; Chiapusio et al., 2004).

The inhibition of germination and particularly the reduction of seedling growth are observed with many plant species exposed to BOA. Often, radicles and root tips are more sensitive than shoots. Generation of ROS (Reactive Oxygen Species) and oxidative stress are key events in the mode of action. As a consequence, membrane damage, lipid peroxidation, and protein oxidation occur (Sanchez-Moreiras et al., 2003; Sanchez-Moreiras and Reigosa, 2005; Singh et al., 2005; Batish et al., 2006). Subsequently, membrane integrity, photosynthesis, electron transport in the mitochondria, and protein synthesis are affected (Burgos et al., 2004;

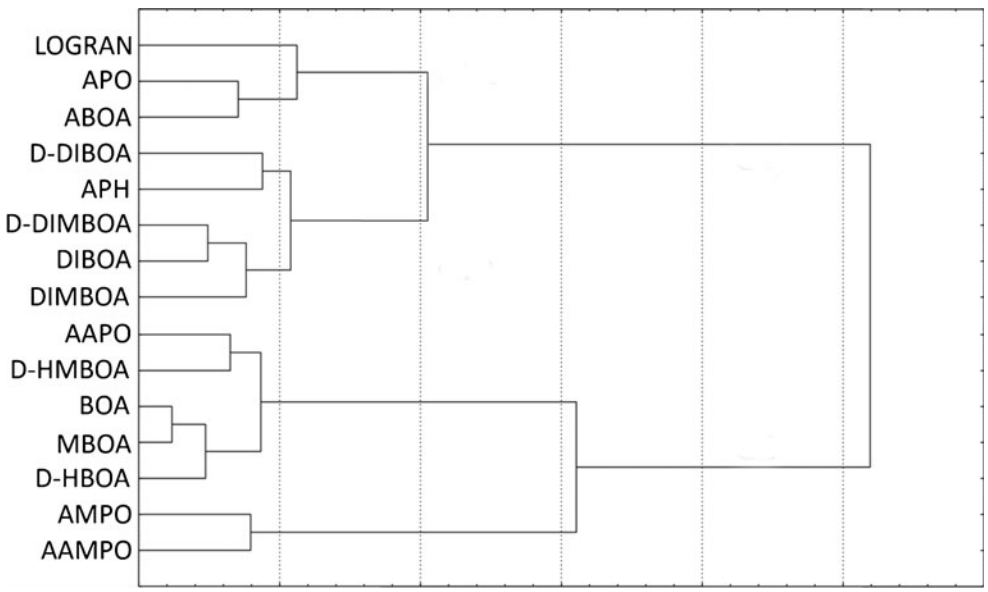


Fig. 4 Cluster analysis for selected allelochemicals, synthetic analogs and degradation products (root and shoot lengths on tested species)

Hussain et al., 2008; Sanchez-Moreiras et al., 2010, 2011; Hussain and Reigosa, 2011;). Lipid metabolism is disrupted, enzymes such as PMH^+-ATPase or α-amylase, are inhibited (Friebe et al., 1997; Burgos et al., 2004; Kato-Noguchi et al., 2010). Cellular transport and the actin cytoskeleton are disturbed (Schulz et al., 2012a). BOA treatment results in the decrease of the densities of ribosomes, dictyosomes, and mitochondria (Burgos et al., 2004). Due to an increased peroxidase activity and H_2O_2 production in BX-treated plants, lignin accumulation and cell wall rigidity are enhanced, which contribute to the growth inhibition (González and Rojas, 1999). Nuclei show shifts in their typical central to lateral and/or axial positions (Schulz et al., 2012a). Benzoxazolinones, known as anti-auxin, can block lateral root formation (Hoshi-Sakoda et al., 1994; Anai et al., 1996; Burgos et al., 2004). Final events are senescence and plant death.

Confronted with sub lethal concentrations, plants activate their rescue and defense program. In *A. thaliana,* about 1 % of the total genes respond with a strong alteration of the expression pattern. Deleterious BOA effects are counteracted by repair and detoxification mechanisms (Baerson et al., 2005). However, the sensitivity to benzoxazinoids depends on plant age, species, and previous stress exposure. For instance, sulfur deficiency leads to a breakdown of BOA detoxification, when additional stresses, such as herbicide treatments, influence maize seedlings (Knop et al., 2007). Monocots often are less sensitive than dicots. According to their genetic, biochemical, and physiological constitution, many weeds in the seedlings stage are sensitive to BOA (Tables 3, 4, and 5); others show almost no stress response.

Moreover, not only different species but also cultivars and ecotypes of one species can exhibit strong differences in their sensitivity, which has to be considered in bioassays for inhibition studies. In one study, *Echinochloa crus-galli* L. was inhibited by BOA, in another one, the species was almost not affected (Nair et al., 1990; Burgos and Talbert, 2000; Macías et al., 2005b). The same is true with *Chenopodium album* ecotypes or different crop cultivars. Maize seems to be able to switch between the different detoxification pathways, depending on the physiological situation (Schulz and Wieland, 1999; Schulz et al., 2012a, b, unpublished). These variations may point to an ongoing adaption of some species to a phytotoxin enriched environment with individual variations in defined populations. Detoxification activities, which are developed in the seedlings, are also dependent on seed and plant age. As a consequence, weed species with ecotypes that are good detoxifiers are less or almost not affected by rye allelochemicals and cannot be controlled by the residues. For instance, the germination of common chickweed [(*Stellaria media* L. (Vill.)] is not inhibited by rye mulch application (Kruidhof et al., 2009).

Although recent work of Rice et al. (2012) indicates that, at most, little amounts of benzoxazolinones remain in the top cm of the soil profile, rye mulch applications have been found to reduce germination and growth of several problematic weeds (Putnam and DeFrank, 1983; Shilling et al., 1985; Hoffman et al., 1996). In a recent study (Tabaglio et al., 2008), rye mulches from several cultivars (Table 2) were not able to suppress velvetleaf and common lambsquarters seedlings, while redroot pigweed and common purslane were significantly affected. However, there was no correlation between total benzoxazinoid amounts and the number of weed seedlings suppressed. The tolerance of *A. theophrasti* to rye mulch leachate agreed with the findings of Hoffman et al. (1996). For *Abutilon theophrasti,* BOA concentrations that occur under natural conditions are not sufficient to suppress seedling growth. Much higher BOA concentrations (Tables 3, 4, and 5) are necessary to achieve growth reduction for this species as it was shown in the study of Burgos and Talbert (2000).

Weed Defense Against Benzoxazinoids - Detoxification Strategies

Since the growth of *Abutilon theophrasti* was not inhibited by BOA, detoxification activities might reduce growth suppression. Four weeds, *Chenopodium album* L., *Amaranthus retroflexus* L., *Abutilon theophrasti,* and *Portulaca oleracea* L., were analyzed in detail for detoxification capacity against a series of low and high BOA concentrations. Exposure to the high BOA concentrations resulted in the production of known BOA detoxification products (Fig. 5). Generally, roots had a higher detoxification activity than shoots. The BOA detoxification products do not accumulate in the plant, as the fraction of the products extractable from the roots is low after several days. At least a portion is exuded by the roots, and can be found in the medium when plants are placed into tap water after BOA incubation (Sicker et al., 2001). BOA-6-O-glucoside was synthesized in all of the species, glucoside carbamate mainly in *P. oleracea, A. retroflexus*, and *C. album.* Malonylglucoside carbamate could be detected in these three species but not in *A. theophrasti.* Traces of gentiobioside carbamate were found in *C. album*. In contrast to the other species, *A. theophrasti* did not accumulate high amounts of free BOA. (Schulz and Wieland, 1999; Wieland et al., 1999; Sicker et al., 2000, 2001, 2003; Sicker and Schulz, 2002; Hofmann et al., 2006; Schulz et al., 2012b) (Table 6).

In addition, germination and seedling growth of the four species were measured after exposure to 0, 0.3, 1.5, 3, 6, and 15 μmol BOA. In contrast to *P. oleracea* and *A. retroflexus*, *A. theophrasti* and *C. album* were only slightly affected by the highest BOA concentrations.

The influence of the MDR (multi drug resistance) transporter inhibitors verapamil, nifedipine, and the GST inhibitor

Table 3 Representative crop plants (root or shoot; root and shoot growth) inhibited by rye residues (RR)/rye extract (RE) and benzoxazinones (BX), benzoxazolinones (BXL) in the given concentrations. CV = cultivar

Crops	RR RE	Highest [BX] used	Highest [BXL] used	Reference
Allium cepa		5 ml, 1.0 mM	5 ml, 1 mM	Macías et al., 2005a, b, c
Avena sativa cv. Jumbo		2 ml, 2 mM	2 ml, 2 mM	Friebe et al., 1997
Lactuca sativa	RE	5 ml, 1 mM 0.3 mg/3 ml	5 ml, 1.0 mM, 0.6 mg/2 ml 1 mM	Macías et al., 2005a, b, c
L. sativa Iceberg				Burgos and Talbert, 2000
L. sativa cv. Great Lakes				Chiapusio et al., 1997
Solanum lycopersicon	RE	5 ml, 1.0 mM 0.3 mg/3 ml	5 ml, 1.0 mM 0.6 mg/2 ml	Macías et al., 2005a, b, c
S. lycopersicon Mt Spring				Burgos and Talbert, 2000
Cucumis melo Mission	RE		1.2 mg/3 ml	Burgos and Talbert, 2000
Cucumis sativum Calypso	RE	0.3 mg/2 ml	1.2 mg/3 ml	Burgos and Talbert, 2000; Burgos et al., 2004
Cucurbito pepo var. *melopepo* Dixie	RE		1.2 mg/3 ml	Burgos and Talbert, 2000
C. pepo Independence II	RR			Walters and Young, 2008
Lepidium sativum		370 µM	5 ml, 1.0 mM	Barnes et al., 1987
Lepidium sativum		5 ml, 1.0 mM		Macías et al., 2005a, b, c
Phaseolus aureus			1–4.7 mg/ml	Singh et al., 2005; Batish et al., 2006
Portulaca oleracea cv. Gelber			0.5 mM, 20 ml/gFW	Hofmann et al., 2006
Raphanus sativus			2 ml, 1 mM	Chiapusio et al., 2004
Vicia faba cv. Alfred			1.0 mM 40 ml/gFW	Wieland et al., 1999

ethacrynic acid on the BOA accumulation and detoxification activity has been studied (Schulz et al., 2012b). All species were mainly affected by nifadipine and ethacrynic acid but in different ways. The most striking result of the inhibitor study was the 3–4 fold higher accumulation of BOA in *A. theophrasti*, which indicates the involvement of highly active transporters and glutathione transferases (GSTs) for extrusion of BOA/detoxification products out of the protoplasts. Similar mechanisms are known from synthetic herbicide detoxification (Conte and Lloyed, 2011). Thus, the four weeds are able to grow in environments with low BOA contents. At higher BOA concentrations, *Abutilon theophrasti* and *Chenopodium album* have a better chance to survive because these species employ mechanisms that avoid the accumulation of BOA (*A. theophrasti*) or contain a strong detoxification system in youngest seedlings (*C. album*). Quite a number of other species have been tested for their BOA detoxification capacity (*Agrostemma githago, Amaranthus albus, Arabidopsis thaliana, Avena fatua, Avena sativa, Capsella bursa-pastoris, Carduus nutans, Centaurea cyanus, Consolida orientalis, Consolida*

Table 4 Representative monocot. plants (root or shoot; root and shoot growth) inhibited by rye residues (RR)/rye extract (RE) and benzoxazinones (BX)/benzoxazolinones (BXL) in the given concentrations

Monocotyledonous weeds				Reference
Avena fatua	RR	5 ml, 1.0 mM	5 ml, 1.0 mM	Osvald, 1953; Peréz 1990
Avena fatua				Macías et al., 2006a, b, c
Dactylis glomerata			100 ml/1.5 mM/70 g perlite	Hussain and Reigosa, 2011
Digitaria saguinalis		1.5 ml, 200 µg	0.6 g/3 ml	Barnes et al., 1987
		0.2 mg		Burgos and Talbert, 2000
Echinochloa crus-galli		200 µg/1.5 ml	5 ml, 1.0 mM	Barnes et al., 1987
		5 ml, 1.0 mM		Macías et al., 2005b
Eleusine indica		0.3 mg/3 ml	0.6 mg/2 ml	Burgos and Talbert, 2000
Lolium perenne			100 ml/1.5 mM/70 g Perlite	Hussain and Reigosa, 2011
Lolium rigidum		5 ml, 1.0 mM	5 ml, 1.0 mM	Macías et al., 2006b
Panicum milaceum		1.5 ml, 200 µg		Barnes et al., 1987

Table 5 Representative dicot. plants (root or shoot; root and shoot growth) inhibited by rye residues (RR)/rye extract (RE) and benzoxazinones (BX)/benzoxazolinones (BXL) in the given concentrations. CV = Cultivar

Dicotyledonous weeds				Reference
Abutilon theophrasti			1 mg/2 ml	Burgos and Talbert, 2000
Ambrosia artemisiifolia	RR			Barnes et al., 1987
Amaranthus retroflexus	RR		40 ml/gFW 5 mM	Schulz et al., 2012a, b; Tabaglio et al., 2008
Amranthus palmeri		0.3 mg/3 ml	0.6 mg/2 ml	Burgos and Talbert, 2000
Arabidopsis thaliana Col-0		1 mM	20–40 ml/gFW, 2 mM	von Rad et al., 2001; Baerson et al., 2005
Chenopodium album	RR		20–40 ml/gFW 5 mM	Schulz and Wieland, 1999; Schulz et al., 2012a, b
Ipomea lacunosa			1 mg/2 ml	Burgos and Talbert, 2000
Portulaca oleracea	RR		20–40 ml/gFW, 5 mM	Schulz et al., 2012a, b; Tabaglio et al., 2008
Rumex acetosa			100 ml/1.5 mM/70 g Perlite	Hussain and Reigosa, 2011
Senna obtusifolia			1 mg/2 ml	Burgos and Talbert, 2000
Sesbania exaltata			1 mg/2 ml	Burgos and Talbert, 2000
Sida spinosa			1 mg/2 ml	Burgos and Talbert, 2000
Taraxacum officinale	RR			Phillips and Young, 1973

regalis, Coriandrum sativum, Daucus carota, Digitaria sanguinalis, Diplotaxis tenuifolia, Galinsoga ciliata, Helianthus annuus, Hordeum vulgare, Legousia speculum veneris, Lolium perenne, Matricaria chamomilla, Papaver rhoeas, Plantago major, Polygonum aviculare, Rhaphanus sativus, Secale cereale, Triticum aestivum, Urtica urens, Vicia faba), (Schulz and Wieland, 1999; Sicker et al., 2003).

As shown above for the four weeds, the plants' strategies to cope with BOA include the rapid activation of detoxification, exudation of phytotoxic compounds and their detoxification products, or an activation of defense mechanisms already in the extracellular matrix (EMC). These mechanisms reveal pronounced differences in their efficiency. In addition, specific (beneficial) microorganisms can participate in mechanisms that lead to a reduced sensitivity to BOA, for instance, by a general strengthening of the plant. Such an interaction seems to be active in *Abutilon theophrasti* roots (Haghikia S., Paetz C., Schneider B. and Schulz M., unpublished). Another possibility is the scavenging of toxic intermediates and radicals.

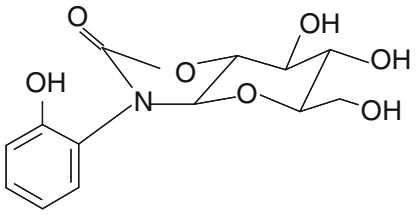
Weed Coexisting with BX-Containg Plants - A Matter of Co-evolution

Except for the BX containing Poacea, which are excellent BOA detoxifiers, detoxification capacity is not necessarily conserved among the species within one plant family. In fact, the affiliation to a defined plant community seems to be much more important. Weed species of the old Secalietea communities have co-existed with wild rye (or wheat) for thousands of years, and may thus have undergone a co-evolutionary process that enables them to share their habitat with BX-containing plants. It is, therefore, not surprising, that species occurring in European ecologically managed rye and wheat fields, such as *Centaurea cyanus, Papaver rhoeas, Legousia speculum-veneris, Consolida regalis,* or *Matricaria chamomilla* can perform BOA detoxification. They are better adapted to BX enriched environments than species of communities without BX containing plants (Schulz and Wieland, 1999; Sicker et al., 2003). It is completely unknown whether these weeds can also have beneficial influences on the crop, perhaps in the defense against pathogens. In manmade agrosystems, adaptation to agrochemicals obviously occurs faster, as underlined by the many weeds which have developed herbicide resistances since the 80s of the last century. Therefore, the use of allelopathy in agriculture demands approaches that decelerate adaptation to allelochemicals. On the other hand, some crops are sensitive to rye residues (Tables 3, 4, and 5). They can develop severe injuries when intercropped with rye, as described for zucchini (*Cucurbita pepo* Independence II), cucumber (*Cucumis sativa*), or snap bean (*Phaseolus vulgaris*) (Chase et al., 1991; Walters and Young, 2008). In contrast, tomato yield was similar or even higher in a tomato cropping system with rye as a cover crop (Masiunas et al., 1995).

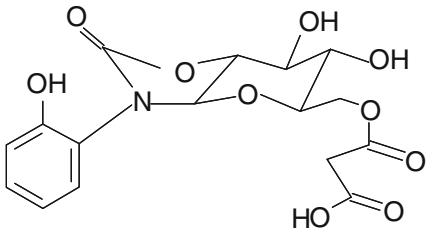
Degradation of Plant Detoxification Products

All plant detoxification products exuded by the roots can be converted to phenoxazinone(s), by fungal and bacterial activities, which most likely localize to the rhizosphere. In maize, phenoxazinone in concentrations of less than 0.3 mM have a stimulatory effect on root growth, but higher concentrations are inhibitory although the compound is obviously not absorbed (Knop et al., 2007; Schulz et al., 2012a). The relatively stable glucoside carbamate is subject to ring opening at the lactone group, which yields glucosylated carbamic acid as

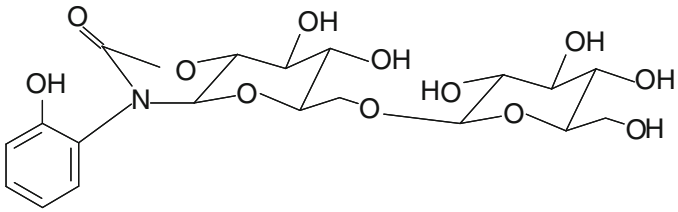
Plant Detoxification products



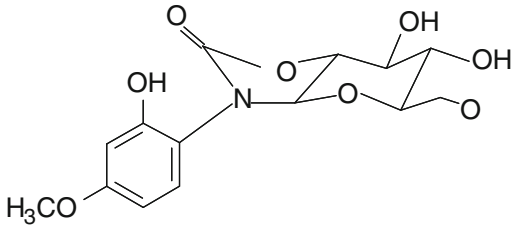
Glucoside carbamate



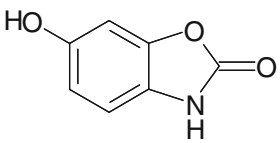
Malonyl glucoside carbamate



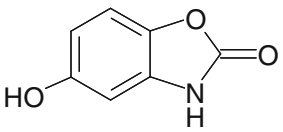
Gentiobioside carbamate



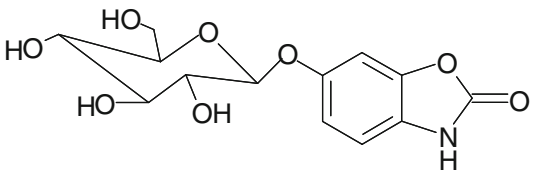
Glucoside methoxycarbamate



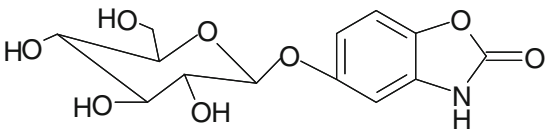
BOA-6-OH



BOA-5-OH

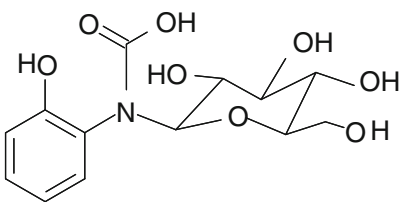


BOA-6-O-glucoside



BOA-5-O-glucoside

Fungal transformation product of glucoside carbamate



Glucosylated carbamic acid

◀ **Fig. 5** Structures of known BOA detoxification products found in higher plants and a fungal transformation product of glucoside carbamate

an intermediate (Schulz et al., 2008; 2012a). Because of the instability of free carbamic acid, subsequent deglucosylation causes either a rearrangement back to BOA, or the aglycone is decarboxylated to 2-aminophenol, the precursor for phenoxazinone production. Some fungi, i.e., *Fusarium graminearum* or *F. oxysporum* (Kobayashi et al., 1998; Brown et al., 2012), secrete lactonohydrolases, which are involved in detoxification reactions (Kimura et al., 2006). Possibly, such enzymes also are able to cleave the glucoside carbamate heterocycle.

BOA-5-O- and -6-O-glucoside can easily be hydrolyzed by common β-glucosidases. BOA-6-OH, which also are intermediates of the detoxification pathway via O-glucoside formation, which is much more toxic than the glucoside (Schulz and Wieland, 1999). Accumulation of highly toxic 6-hydroxylated BOA is, therefore, characteristic for BOA sensitive species. Hydroxylated BOA molecules also are substrates for heterocyclic ring opening by certain fungi, such as *Fusarium solani*, and production of phenoxazinones (Schulz et al., unpublished).

As already mentioned, phenoxazinones are toxic for many plants, fungi, and bacteria (Knop et al., 2007, and see above). At least some specialized fungi, for instance, *Fusarium* species, destroy the reducting agent phenoxazinone via the Fenton reaction, if the chemical environment, such as pH and Fe^{2+} ion concentrations, is suitable (Schulz et al., 2012a). Those fungi excrete H_2O_2 or oxalate that initiates the destruction of the phenoxazinone skeleton in the presence of Fe^{2+}. Quite a number of fungi are known to excrete H_2O_2/oxalate under certain conditions, dependent on substrate availablitiy, pathogenicity of the fungus, and stress (Dutton and Evans, 1996; Cessna et al., 2000). Oxalate can facilitate hydroxyl radical formation (Varela and Tien, 2003). The importance of the Fenton reaction for organic molecule destruction in natural environments is increasingly recognized (Vlyssides et al., 2011). The capability of soil fungi to degrade phenoxazinones by the Fenton reaction in the aerated soil is thus of high importance to fulfill several of the major prerequisites for the use of benzoxazinoids as bioherbicides: a selective efficiency, biodegradability, and therefore a short half life in the environment. However, more research in this field has to be done.

Allelopathic Weed Management

Weed management is the most difficult challenge for organic producers (Wallace, 2001; Teasdale et al., 2004; Liebman and Davis, 2009). Simply replacing synthetic herbicides by other direct control measures is inadequate. Instead, weed management should be seen as a component of integrated crop management, with the need for a deep integration with the other cultural practices, to optimize the entire cropping system rather than the weed control *per se* (Bàrberi, 2002). In organic farming systems, weeds are controlled through crop rotation, cover crops, mechanical means, primary and secondary cultivation, hand weeding, flaming, biocontrol, weed seed predation, smoother crops, competition, natural herbicides, and allelopathy (Liebman and Gallandt, 1997; Wallace, 2001; Liebman and Davis, 2009; Kalinova, 2010; Flamini, 2012).

Cover crops fit well into such an integrated approach, as they provide many additional services to the agro-ecosystem, including improvement of soil structure and water infiltration, reduction of soil erosion, increase of soil fertility and nutrient cycling, reduction of soil nutrient losses due to leaching, enhancement of biodiversity, and contribution to weed and pest management (Altieri, 1987; Sarrantonio and Gallandt,

Table 6 BOA-detoxification products in plants and degradation of glucoside carbamate

Detoxification product	Occurrence- first description	Reference
BOA-6-O-β-D-glucoside	Dicots/monocots	Wieland et al., 1999
	Avena sativa	Sicker et al., 2003
BOA-5-O-β-D-glucoside	*Portulaca oleracea*	Hofmann et al., 2006
Glucoside carbamate (from BOA-N-β-D-glucoside)	Mainly monocots *(Secale cereale, Zea mays,)* some dicots	Wieland et al., 1998
	Avena sativa	Sicker et al., 2003
Gentiobioside carbamate	*Zea mays* *Chenopodium album ec.*	Sicker et al., 2001
Malonyl-glucoside carbamate	*Zea mays* *Portulaca oleracea*	Hofmann et al., 2006
Glucoside methoxycarbamate	*Zea mays*	Hofmann et al., 2006
N-ß-D-glucopyranosyl-N-(2′-hydroxyphenyl) carbamic acid, fungal degradation product of plant produced glucoside carbamate	*Fusarium verticillioides* *F. oxysporum* and others	Schulz et al., 2012a

2003; Clark, 2007). Some cover crops produce allelochemicals, and they can exert a strong influence on weeds through the release of chemicals from living or dead plant tissue (Liebman and Davis, 2009).

Rye'S Weed Suppressive Potential in Organic Farming

Rye is one of the best cool season cover crop and widely used for its high biomass production, earliness, wide soil and climate adaptability, and exceptional weed suppression potential (Masiunas et al., 1995; Batish et al., 2001; Clark, 2007; Tabaglio et al., 2008; Gavazzi et al., 2010). Rye effectively suppresses weeds by shading, competition, and allelopathy. Allelopathic suppression can occur while the rye crop is living, due to the thick and tall stand. At termination of the cover crop, chemicals are released from the dead mulch produced by mowing, chopping, rolling, or spraying (not allowed in organic farming) the biomass. The mulch can be left on the soil surface, to ensure a stronger and more persistent inhibitory effect, or tilled in by disking or plowing. The level of weed suppression also depends on the thickness of the mulch layer, with an exponential relationship between mulch mass and weed emergence (Teasdale and Mohler, 2000). Mulching has other favorable mechanisms to suppress weeds in the field. Residues left on the soil surface can lead to decreased soil temperature fluctuations and reduced light penetration, which both have been shown to inhibit weed germination (Teasdale and Mohler, 1993; Liebman and Mohler, 2001). Furthermore, in some cases, soil microbial populations, including soil pathogens, are either stimulated (Dabney et al., 1996; Conklin et al., 2002; Manici et al., 2004) or suppressed (Matthiessen and Kirkegaard, 2006) after soil amendment with fresh residue material.

Figure 6 demonstrates that rye mulch significantly reduced the germination and growth emergence of several problematic agronomic grass and broadleaf weeds. These includes common ragweed (*Ambrosia artemisiifolia* L.), eastern black night shade (*Solanum ptycanthum* Dum.), bermuda grass [*Cynodon dactylon* (L.) Pers.], crabgrass (*Digitaria* spp.), barnyardgrass (*Echinochloa crus-galli* L.), yellow and green foxtail (*Setaria* spp. L. Beauv.], and the weed species already mentioned. (Putnam and DeFrank, 1983; Shilling et al., 1985; Narwal, 1994; Khanh et al., 2005; Tabaglio et al., 2008; Gavazzi et al., 2010; Narwal, 2010; Schulz et al., 2012b). In particular, Gavazzi et al. (2010) found that grass weeds were reduced by 61 %, whereas broadleaf were reduced by 96 % when rye mulch was used in a no-tillage system. These findings agree with those of Barnes and Putnam (1987) who found that broadleaf weeds were approximately 30 % more sensitive to DIBOA and BOA compared with grass weeds. Other authors have confirmed a lower sensibility for grass weeds, but with very different values (Nagabhushana et al., 2001; Tet-Vun and Ismail, 2006). Further reports have shown that larger-seeded species are less sensitive to allelochemicals (Weidenhamer et al., 1987; Chase et al., 1991; Tabaglio et al., 2008) and that seed mass is particularly important for the selective suppression of weeds with crop residues (Mohler, 1996; Liebman and Davis, 2000).

Kruidhof et al. (2010) found that seed mass and time of emergence significantly contributed to the variance in target plant emergence. In particular, this study indicated that residue-mediated inhibition of a receptor plant only takes place when there is an overlap of the time course of sensitivity of the receptor plant and the time course of the residue-mediated inhibitory potential.

Crop residues left on the soil surface decompose more slowly than residues incorporated into the soil, which may result in a slower release rate but longer lasting supply of allelochemicals (Kruidhof et al., 2009). When the residue material is retained on the soil surface, effective weed control can be observed up to 4 to 8 weeks after mulching (Smeda and Weller, 1996; Ercoli et al. 2005; Gavazzi et al., 2010). Alternatively, the cover crop can be incorporated into the soil directly or after chopping. This treatment increases the decomposition rate and thereafter the release rate of allelochemicals (Angers and Recous, 1997). The incorporation may explain the different results reported by Krogh et al. (2006) in comparison to Rice et al. (2012) and Teasdale et al. (2012).

Fig. 6 Field trial on allelopathic cover crops preceding a tomato crop in a biological farm. *Left*, plot with rye mulch left on the soil surface, showing the good weed suppression ability. *Right*, control plot without cover crop, split in two treatments: *left side*, untreated sub-plot in which tomato plants are almost completely overgrown by weeds; *right side*, sub-plot with mechanical control by cultivations

Recent Practices and Future Strategies

The study of pure stand cover crops allows obtaining detailed information on the effect of a particular species; however, various combinations of cover crops might provide additional agronomic advantages. Altieri et al. (2011) assessed the effects of various combinations of rye (*Secale cereale*), vetch (*Vicia villosa*), fodder radish (*Raphanus sativus subsp. oleiferus*), black oats (*Avena strigosa*), and ryegrass (*Lolium multiflorum*) in reducing winter and summer weed populations in bean crops. Results indicate that the best cover crop mixtures should include a significant proportion of rye, vetch, and fodder radish. Moreover, Worsham (1991) used a mixture of rye and *Trifolium subterraneum* and noted a reduction of 80–90 % sicklepod (*Cassia obtusifolia* L.), morning glory (*Ipomoea* spp.), prickly sida (*Sida spinosa* L.), and pigweed (*Amaranthus* spp.) in soybean, tobacco, maize, sorghum, and sunflower. The main advantages of mixtures are: higher biomass, larger spectrum of target weeds, wider adaptability to pedo-climatic conditions, complementary effects on soil quality (N-fixation for legumes, nematocidal effect and improving soil tilth for brassicas, nitrate scavenging and building soil structure for grasses).

In organic farming, weed management is based on a combination of agronomic practices, including a false seedbed approach to deplete seed banks, consisting in repeated cultivations before planting. For instance, Kruidhof et al. (2008) in field experiments carried out in a biological experimental farm in the Netherlands, reported that delayed sowing of winter rye cover crop in combination with a stale seedbed can severely reduce weed pressure more than five times. On the other hand, a later sowing date also implies a reduced amount of residue that can be incorporated in spring.

The strategy to use allelochemical extracts as natural herbicides represents a future challenge. Several researchers have suggested the use of aqueous extracts, which have provided excellent results in laboratory studies, also in applications under field conditions (Ercoli et al., 2007). However, the identification of a plant-based chemical that shows allelopathic properties in the laboratory represents only the first step towards the development of an agronomically relevant weed control system. It is furthermore required that this chemical suppress weed growth in a farming system, and also that the chemical can be economically extracted and used as a natural herbicide (Breen and Ogasawara, 2011). From an agronomic perspective, selectivity between crop and weeds is another important consideration that should be addressed. The management of a rye cover crop or the use of bioherbicides must be optimized to provide maximized weed suppression, but must not interfere with crop production. In addition, high residue biomass may facilitate weed suppression, but interfere with planting and establishment of the crop, or with crop growth by raising the soil C/N ratio, leading to unavailability of nitrogen to crops.

In summary, it is useful to add rye cover crops, in pure stand or mixtures, to a kit for a weed management strategy in organic farming, even if the efficacy and the extent of the suppressive effect are not guaranteed. A better understanding of allelopathic effects in field situations (soil, climate and agronomic conditions), and of the dependency on cultural practices (cultivar, mixtures, fertilization level, conventional tillage or no till systems, timing, and system of termination) would provide the means to profitably include rye cover in organic cropping systems and use it as a complementary strategy in weed management. Advisors and farmers should recognize the importance of individual field and farm analyses to develop site-specific, farm-adapted weed management strategies in organic farming (Lundkvist et al., 2008).

Perspectives

The present knowledge of rye allelopathy shows some striking deficits that should be considered in future research. Systematic screenings for crop detoxification strategies and their differences in cultivars will help to unravel the different detoxification pathways in plants and microbes. Rye cultivar dependent variations of BX compositions in root exudates have rarely been investigated, and the pathways of exudation are not known, nor is the degree of long distance transport of benzoxazinones. The strategies of weeds to cope with benzoxazinoids combined with the genetic background of these strategies have not been studied, except for *Arabidopsis*. These questions should be addressed if rye allelopathy is to be used more commonly in the future. Another necessary focus is the protection of the environment, which demands further research on the degradability of phenoxazinones. Some specific considerations for further research needs are listed below:

- The detoxification potentials of crop cultivars should be important traits for breeding strategies.
- BX contents in rye cultivars need to be systematically analyzed, as well as the dependence of BX contents on environmental factors. These two parameters are prerequisites for breeding of high BX rye varieties as cover crop. Such breeding programs already have been started by US researchers (see review of Worthington and Reberg-Horton, 2013).
- The use of rye mulching in sustainable agroecosystems has to be optimized by studying more intensively BX accumulation patterns in rye plants during growth in order to choose the best time point for the cover crop termination.

- Aspects of agroecology must be considered, such as: avoidance of fast weed adaption, which should include research in population genetics; the maintenance of a high diversity of soil microorganisms; plant signaling that is important for herbivore defense (Degenhardt et al., 2009); and beneficial plant-plant interactions.
- Additional research is necessary to provide unequivocal evidence for BX and, even more important, phenoxazinone biodegradability in the soil, which is not only a requirement for organic farming and for optimizing soil management, but is of general importance for the protection of the environment. Such studies should be combined with research on weed suppression and with systematic screenings to identify primary and secondary effects of the mode of action. In the past, this important area either has been ignored or has not received sufficient attention.

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References

AHMAD, S., VEYRAT, N., GORDON-WEEKS, R., ZHANG, Y., MARTIN, J., SMART, L., GLAUSER, G., ERB, M., FLORS, V., FREY, M., and TON, J. 2011. Benzoxazinoid metabolites regulate innate immunity against aphids and fungi in maize. *Plant Physiol.* 157:317–327.

ALTIERI, M. A. 1987. Cover cropping and mulching, pp. 219–232, *in* M. A. Altieri (ed.), Agroecology: The Science of Sustainable Agriculture, 2nd ed. Westview Press, Boulder, CO, USA.

ALTIERI, M. A., LANA, M. A., BITTENCOURT, H. V., KIELING, A. S., COMIN, J. J., and LOVATO, P. E. 2011. Enhancing crop productivity via weed suppression in organic no-till cropping systems in Santa Catarina, Brazil. *J. Sustain. Agric.* 35:855–869.

ANAI, T., AIZAWA, H., OHTAKE, N., KOSEMURA, S., YAMAMURA, S., and HASEGAWA, K. 1996. A new auxin inhibiting substance, 4-Cl-6,7-dimethoxy-2-benzoxazolinone, from light-grown maize shoots. *Phytochemistry* 42:273–275.

ANDREASEN, M. F., CHRISTENSEN, L. P., MEYER, A. S., and HANSEN, A. 2000. Content of phenolic acids and ferulic acid dehydrodimers in 17 rye (*Secale cereale* L.) varieties. *J. Agric. Food Chem.* 48:2837–2842.

ANGERS, D. A. and RECOUS, S. 1997. Decomposition of wheat straw and rye residues as affected by particle size. *Plant Soil* 189:197–203.

ARGANDOÑA, V. H., LUZA, J. G., NIEMEYER, H. M., and CORCUERA, L. J. 1980. Role of hydroxamic acids in the resistance of cereals to aphid. *Phytochemistry* 19(1665):1668.

BACON, C. W., HINTON, D. M., GLENN, A. E., MACÍAS, F. A., and MARIN, D. 2007. Interactions of *Bacillus mojavensis* and *Fusarium verticillioides* with a benzoxazolinone (BOA) and its transformation product, APO. *J. Chem. Ecol.* 33:1885–1897.

BAERSON, S. R., SANCHEZ-MOREIRAS, A. M., PEDROL-BONJOCH, N., SCHULZ, M., KAGAN, I. A., AGARWAL, A. K., REIGOSA, M. J., and DUKE, S. O. 2005. Detoxification and transcriptome response in *Arabidopsis* seedlings exposed to the allelochemical benzoxazolin-2(3*H*)-one. *J. Biol. Chem.* 280:21867–21881.

BÀRBERI, P. 2002. Weed management in organic agriculture: are we addressing the right issues? *Weed Res.* 42:177–193.

BARNES, J. P. and PUTMAN, A. R. 1983. Rye residues contribute weed suppression in no-tillage cropping systems. *J. Chem. Ecol.* 9:1045–1057.

BARNES, J. P. and PUTNAM, A. R. 1986. Evidence for allelopathy by residues and aqueous extracts of rye (*Secale cereale* L.). *Weed Sci* 34:384–390.

BARNES, J. P. and PUTNAM, A. R. 1987. Role of benzoxazinones in allelopathy by rye (*Secale cereale* L.). *J. Chem. Ecol.* 56:1788–1800.

BARNES, J. P., PUTNAM, A. R., BURKE, B. A., and AASEN, A. J. 1987. Isolation and characterization of allelochemicals in rye herbage. *Phytochemistry* 26:1385–1390.

BATISH, D. R., SINGH, H. P., KOHLI, R. K., and KAUR, S. 2001. Crop allelopathy and its role in ecological agriculture. *J. Crop. Prod.* 4:121–162.

BATISH, D. R., SINGH, H. P., SETIA, N., KAUR, S., and KOHLI, R. K. 2006. 2-Benzoxazolinone (BOA) induced oxidative stress, lipid peroxidation and changes in some antioxidant enzyme activities in mung bean (*Phaseolus aureus*). *Plant Physiol. Biochem.* 44:819–827.

BELZ, R. G. 2007. Allelopathy in crop/weed interactions—an update. *Pest Manag. Sci.* 63:308–326.

BENINGER, C. W. and HALL, J. C. 2005. Allelopathic activity of luteolin 7-O-β-glucuronide from *Chrysanthemum morifolium* L. *Biochem. Syst. Ecol.* 33:103–111.

BERTHOLDSSON, N. 2010. Breeding spring wheat for improved allelopathic potential. *Weed Res.* 50:49–57.

BERTHOLDSSON, N.-O., ANDERSSON, S. C., and MERKER A. 2012. Allelopathic potential of *Triticum* spp., *Secale* spp. and *Triticosecale* spp. and use of chromosome substitutions and translocations to improve weed suppression ability in winter wheat. *Plant Breeding* 131:75–80.

BOOKER, F. L., BLUM, U., and FISCUA, E. L. 1992. Short-term effects of ferulic acid on uptake and water relations in cucumber seedlings. *J. Exp. Bot.* 43:649–655.

BRAVO, H. R. and NIEMEYER, H. M. 1985. Decomposition in aprotic solvents of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one, a hydroxamic acid from cereals. *Tetrahedron* 41:4983–4986.

BREDENBERG, J. B., HONKANEN, E., and VIRTANEN, A. I. 1962. The kinetics and mechanism of the decomposition of 2,4-dihydroxy-1,4-benzoxazin-3-one. *Acta Chem. Scand.* 16:135–141.

BREEN, J. and OGASAWARA, M. 2011. A vision for weed science in the twenty-first century. *Weed Biol. Manag.* 11:113–117.

BROOKS, A. M., DANEHOWER, D. A., MURPHY, J. P., REBERG-HORTON, S. C., and BURTON, J. D. 2012. Estimation of heritability of benzoxazinoid production in rye (*Secale cereale*) using gas chromatographic analysis. *Plant Breeding* 131:104–109.

BROWN, N. A., ANTONIW, J., and HAMMOND-KOSACK, K. E. 2012. The predicted secretome of the plant pathogenic fungus *Fusarium graminearum*: a refined comparative analysis. *PLoS One* 7(4): e33731. doi:10.1371/journal.pone.0033731.

BURGOS, N. R. and TALBERT, R. E. 2000. Differential activity of allelochemicals from *Secale cereale* in seedling bioassays. *Weed Sci.* 48:302–310.

BURGOS, N. R., TALBERT, R. E., and MATTICE, J. D. 1999. Cultivar and age differences in the production of allelochemicals by *Secale cereale*. *Weed Sci.* 47:481–485.

BURGOS, N. R., TALBERT, R. E., KIM, K. S., and KUK, Y. I. 2004. Growth inhibition and root ultrastructure of cucumber seedlings exposed to allelochemicals from rye (*Secale cereale*). *J. Chem. Ecol.* 30:671–689.

CESSNA, S. G., SEARS, V. E., DICKMAN, M. B., and LOW, P. B. 2000. Oxalic acid, a pathogenicity factor for *Sclerotinia sclerotiorum*, suppresses the oxidative burst of the host plant. *Plant Cell* 12:2191–2199.

CHASE, W. R., NAIR, M. G., and PUTNAM, A. R. 1991. 2,2'-oxo-1, 1'-azobenzene: selective toxicity of rye (*Secale cereale* L.) allelochemicals to weed and crop species: II. *J. Chem. Ecol.* 17:9–19.

CHEN, K.-J., ZHENG, Y.-Q., KONG, C.-H., ZHANG, S.-Z., LI, J., and LIU, X.-G. 2010. 2,4-Dihydrox-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and 6-methoxy-benzoxazolin-2-one (MBOA) levels in the wheat rhizosphere and their effect on the soil microbial community structure. *J. Agric. Food Chem.* 58:12710–12716.

CHEN, L., YANG, X., RAZA, W., LI, J., LIU, Y., QIU, M., ZHANG, F., and SHEN, Q. 2011. *Trichoderma harzianum* SQR-T037 rapidly degrades allelochemicals in rhizospheres of continuously cropped cucumbers. *Appl. Microbiol. Biotechnol.* 89:1653–1663.

CHIAPUSIO, G., SÁNCHEZ, A. M., REIGOSA, M. J., GONZÁLEZ, L., and PELLISSIER, F. 1997. Do germination indices adequately reflect allelochemical effects on the germination process? *J. Chem. Ecol.* 23:2445–2453.

CHIAPUSIO, G., PELLISSIER, F., and GALLET, C. 2004. Uptake and translocation of phytochemical 2-benzoxazolinone (BOA) in radish seeds and seedlings. *J. Exp. Bot.* 55:1587–1592.

CHIKMAWATI, T., SKOVMAND, B., and GUSTAFSON, J. P. 2005. Phylogenetic relationships among *Secale* species revealed by amplified fragment length polymorphisms. *Genome* 48:792–801.

CICEK, M., BLANCHARD, D., BEVAN, D. R., and ESEN, A. 2000. The aglycone specificity-determining sites are different in 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA)-glucosidase (maize beta-glucosidase) and dhurrinase (sorghum beta-glucosidase). *J. Biol. Chem.* 275:20002–20011.

CIPOLLINI, D., RIGSBY, C. M., and BARTO, E. K. 2012. Microbes as targets and mediators of allelopathy in plants. *J. Chem. Ecol.* 38:714–727.

CLARK, A. 2007 (ed.). Managing Cover Crops Profitably, p. 244. Sustainable Agriculture Network, Handbook Series Book 9, 3rd ed., Beltsville, MD, USA.

COJA, T., IDINGER, J., and BLUEMEL, S. 2006. Effects of the benzoxazolinone BOA, selected degradation products and structure related pesticides on soil organisms. *Ecotoxicology* 15:61–72.

COLLANTES, H. G., GIANOLI, E., and NIEMEYER, H. M. 1999. Defoliation affects chemical defenses in all plant parts of rye seedlings. *J. Chem. Ecol.* 25:491–499.

CONKLIN, A. E., ERICH, M. S., LIEBMAN, M., LAMBERT, D., GALLANDT, E. R., and HALTEMAN, W. A. 2002. Effects of red clover (*Trifolium pratense*) green manure and compost soil amendments on wild mustard (*Brassica kaber*) growth and incidence of disease. *Plant Soil* 238:245–256.

CONTE, S. S. and LLOYED, A. M. 2011. Exploring multiple drug and herbicide resistance in plants-spotlight on transporter proteins. *Plant Sci.* 180:196–203.

COPAJA, S. V., VILLARROEL, E., BRAVO, H. R., PIZARRO, L., and ARGANDOÑA, V. H. 2006. Hydroxamic acids in *Secale cereale* L. and the relationship with their antifeedant and allelopathic properties. *Z. Naturforsch. C* 61:670–676.

DABNEY, S. M., SCHREIBER, J. D., ROTHROCK, C. S., and JOHNSON, J. R. 1996. Cover crops affect sorghum seedling growth. *Agron. J.* 88:961–970.

DE BRUIN, J. L., PORTER, P. M., and JORDAN, N. R. 2005. Use of a rye cover crop following corn in rotation with soybean in the upper midwest. *Agron. J.* 97:587–598.

DEGENHARDT, J., HILTPOLD, I., KÖLLNER, T. G., FREY, M., GIERL, A., GERSHENZON, J., HIBBARD, B. E., ELLERSIECK, M. R., and TURLINGS, T. C. J. 2009. Restoring a maize root signal that attracts insect-killing nematodes. *Proc. Natl. Acad. Sci. U. S. A.* 106:13213–13218.

DELLAMONICA, G., MEURER, B., STRACK, D., WEISSENBÖCK, G., and CHOPIN, J. 1983. Two isovitexin 2″-*O*-glycosides from primary leaves of *Secale cereale*. *Phytochemistry* 22:2627–2628.

DICK, R., RATTEI, T., HASLBECK, M., SCHWAB, W., GIERL, A., and FREY, M. 2012. Comparative analysis of benzoxazinoid biosynthesis in monocots and dicots: independent recruitment of stabilization and activation functions. *Plant Cell* 24:915–928.

DUFALL, L. A. and SOLOMON, P. S. 2011. Role of cereal secondary metabolites involved in mediating the outcome of plant-pathogen-interaction. *Metabolites* 1:64–78.

DUTARTRE, L., HILIOU, F., and FEYEREISEN, R. 2012. Phylogenomics of the benzoxazinoid biosynthetic pathway of Poaceae: gene duplications and origin of the BX cluster. *BMC Evol. Biol.* 12:64–83.

DUTTON, M. V. and EVAN, C. S. 1996. Oxalate production by fungi: its role in pathogenicity and ecology in the soil environment. *Can. J. Microbiol.* 42:881–895.

EBISUI, K., ISHIHARA, A., and IWAMURA, H. 2001. Purification and characterization of UDP-glucose: cyclic hydroxamic acid-glucosyltransferases from maize seedlings. *Plant Physiol. Biochem.* 39:27–35.

EHLERS, B. K. 2011. Soil microorganisms alleviate the allelochemical effects of a thyme monoterpene on the performance of an associated grass species. *PLoS One* 6:e26321.

ERCOLI, L., MASONI, A., and PAMPANA, S. 2005. Weed suppression by winter cover crops. *Allelopathy J.* 16:273–278.

ERCOLI, L., MASONI, A., PAMPANA, S., and ARDUINI, I. 2007. Allelopathic effects of rye, brown mustard and hairy vetch on redroot pigwee, common lambsquarter and knotweed. *Allelopathy J.* 19:249–256.

FINNEY, M. M., DANEHOWER, D. A., and BURTON, J. D. 2005. Gas chromatographic method for the analysis of allelopathic natural products in rye (*Secale cereale* L.). *J. Chromatogr.* 1066:249–253.

FLAMINI, G. 2012. Natural herbicides as a safer and more environmentally friendly approach to weed control: a review of the literature since 2000. *Stud. Nat. Prod. Chem.* 38:353–396.

FREY, M., CHOMET, P., GLAWISCHNIG, E., STETTNER, C., GRÜN, S., WINKLMAIR, A., EISENREICH, W., BACHER, A., MEERLEY, R. B., BRIGGS, S. P., SIMCOX, K., and GIERL, A. 1997. Analysis of a chemical plant defense mechanism in grasses. *Science* 277:696–699.

FREY, M., STETTNER, C., PARE, P. W., SCHMELZ, E. A., TUMLINSON, J. H., and GIERL, A. 2000. An herbivore elicitor activates the gene for indole emission in maize. *Proc. Natl. Acad. Sci. U. S. A.* 97:14801–14806.

FREY, M., HUBER, K., PARK, W. J., SICKER, D., LINDBERG, P., MEELEY, R. B., SIMMONS, C. R., YALPANI, N., and GIERL, A. 2003. A 2-oxoglutarate-dependent dioxygenase is integrated in DIMBOA biosynthesis. *Phytochemistry* 62:371–376.

FREY, M., SPITELLER, D., BOLAND, W., and GIERL, A. 2004. Transcriptional activation of Igl, the gene for indole formation in *Zea mays*: a structure-activity study with elicitor-active N-acyl glutamines from insects. *Phytochemistry* 65:1047–1055.

FREY, M., SCHULLEHNER, K., DICK, R., FIESSELMANN, A., and GIERL, A. 2009. Benzoxazinoid biosynthesis, a model for evolution of secondary metabolic pathways in plants. *Phytochemistry* 70:1645–1651.

FRIEBE, A., WIELAND, I., and SCHULZ, M. 1996. Tolerance of *Avena sativa* to the allelochemical benzoxazolinone. Degradation of BOA by root-colonizing bacteria. *J. Appl. Bot.-Angew. Bot.* 70:150–154.

FRIEBE, A., ROTH, U., KÜCK, P., SCHNABL, H., and SCHULZ, M. 1997. Effects of 2,4-dihydroxy-1,4-benzoxazin-3-ones on the activity of plasma membrane H^+-ATPase. *Phytochemistry* 44:979–983.

FRIEBE, A., VILICH, V., HENNIG, L., KLUGE, M., and SICKER, D. 1998. Detoxification of benzoxazolinone allelochemicals from wheat by *Gaeumannomyces graminis* var. tritici, *G. graminis* var. graminis, *G. graminis* var. avenae, and *Fusarium culmorum*. *Appl. Environ. Microb.* 64:2386–2391.

FRITZ, J. I. and BRAUN, R. 2006. Ecotoxicological effects of benzoxazinone allelochemicals and their metabolites on aquatic nontarget organisms. *J. Agric. Food Chem.* 54:1105–1110.

GAGLIARDO, R. W. and CHILTON, W. S. 1992. Soil transformation of 2(3*H*)-benzoxazolinone of rye into phytotoxic 2-amino-3*H*-phenoxazin- 3-one. *J. Chem. Ecol.* 18:1683–1691.

GAVAZZI, C., SCHULZ, M., MAROCCO, A., and TABAGLIO, V. 2010. Sustainable weed control by allelochemicals from rye cover crops: from the greenhouse to field evidence. *Allelopathy J.* 25:259–273.

GEALY, D. R., GURUSIDDAIAH, S., and OGG JR., A. G. 1996. Isolation and characterization of metabolites from *Pseudomonas syringae*-strain 3366 and their phytotoxicity against certain weed and crop species. *Weed Sci.* 44:383–392.

GENTS, M. B., NIELSEN, S. T., MORTENSEN, A. G., CHRISTOPHERSEN, C., and FOMSGAARD, I. S. 2005. Transformation products of 2-benzoxazolinone (BOA) in soil. *Chemosphere* 61:74–84.

GIANOLI, E., RIOS, J. M., and NIEMEYER, H. M. 1999. Factors governing within-plant allocation of a chemical defence in *Secale cereale*. Which is the appropriate currency of allocation? *Chemoecology* 9:113–117.

GLAUSER, G., MARTI, G., VILLARD, N., DOYEN, G. A., WOLFENDER, J. L., TURLINGS, T. C. J., and ERB, M. 2011. Induction and detoxification of maize 1,4-benzoxazin-3-ones by insect herbivores. *Plant J.* 68:901–911.

GLAWISCHNIG, E., GRÜN, S., FREY, M., and GIERL, A. 1999. Cytochrome P450 monooxygenase of DIBOA biosynthesis: specificity and conservation among grasses. *Phytochemistry* 50:925–930.

GLENN, A. E., HINTON, D. M., YATES, I. E., and BACON, C. W. 2001. Detoxification of corn antimicrobial compounds as the basis for isolations *Fusarium verticillioides* and some other *Fusarium* species from Corn. *Appl. Environ. Microbiol.* 67:2973–2981.

GONZÁLEZ, L. F. and ROJAS, M. C. 1999. Role of wall peroxidases in oat growth inhibition by DIMBOA. *Phytochemistry* 50:931–937.

GRAYSTON, S. J., WANG, S. A., CAMPBELL, C. D., and ADWARDS, A. C. 1998. Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biol. Biochem.* 30:369–378.

HARTENSTEIN, H. and SICKER, D. 1994. (2*R*)-2-β-D-glucopyranosyloxy-4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one from S*ecale cereale*. *Phytochemistry* 35(827):828.

HASHIMOTO, Y. and SHUDO, K. 1996. Chemistry of biologically active benzoxazinoids. *Phytochemistry* 43:551–559.

HIETALA, P. K. and VIRTANEN, A. I. 1960. Precursors of benzoxazolinone in rye plants. II. Precursor I, the glucoside. *Acta Chem. Scand. A* 14:502–504.

HOFFMAN, M. L., WESTON, L. A., SNYDER, J. C., and REGNIER, E. R. 1996. Separating the effects of sorghum *(Sorghum bicolor*) and rye (*Secale cereale*) root and shoot residues on weed development. *Weed Sci.* 44:402–407.

HOFMAN, J. and HOFMANOVA, O. 1969. 1,4-Benzoxazinone derivatives in plants. *Eur. J. Biochem.* 8:109–112.

HOFMANN, D., KNOP, M., HAO, H., HENNIG, L., SICKER, D., and SCHULZ, M. 2006. Glucosides from MBOA and BOA detoxification by *Zea mays* and *Portulaca oleracea*. *J. Nat. Prod.* 69:34–37.

HOSHI-SAKODA, M., USU, K., ISHIZUKA, K., KOSEMURA, S., YAMAMURA, S., and HASEGAWA, K. 1994. Structure-activity relationships of benzoxazolinones with respect to auxin-induced growth and auxin-binding protein. *Phytochemistry* 37:297–300.

HUSSAIN, M. I. and REIGOSA, M. J. 2011. Allelochemical stress inhibits growth, leaf water relations, PSII photochemistry, non-photochemical fluorescence quenching, and heat energy dissipation in three C_3 perennial species. *J. Exp. Bot.* 62:4533–4545.

HUSSAIN, M. I., GONZALEZ, L., and REIGOSA, M. J. 2008. Germination and growth response of four plant species towards different allelochemicals and herbicides. *Allelopathy J.* 22:101–110.

IDINGER, J., COJA, T., and BLÜMEL, S. 2006. Effects of the benzoxazoid DIMBOA, selected degradation products, and structure-related pesticides on soil organisms. *Ecotoxicol. Environ. Saf.* 65:1–13.

JILANI, G., MAHMOOD, S., CHAUDHRY, A. N., HASSAN, I., and AKRAM, M. 2008. Allelochemicals: sources, toxicity and microbial transformation in soil—a review. *Ann. Microbiol.* 58:351–357.

JONCZYK, R., SCHMIDT, H., OSTERRIEDER, A., FISSELMANN, A., SCHULLEHNER, K., HASLBECK, M., SICKER, D., HOFMANN, D., YALPANI, N., and SIMMONS, C. 2008. Elucidation of the final reactions of DIMBOA-glucoside biosynthesis in maize: characterization of *Bx6* and *Bx7*. *Plant Physiol.* 146:1053–1063.

KALINOVA, J. 2010. Allelopathy and organic farming, pp 379–418, *in* E. Lichtfouse (ed.), Sociology, Organic Farming, Climate. Change and Soil Science. Sustainable Agriculture Reviews 3, Springer Science+Business Media B.V.

KATO-NOGUCHI, H., and PETERS, R.J. 2013. The role of momilactones in rice allelopathy. *J. Chem. Ecol.* 39:175–185.

KATO-NOGUCHI, H., MACIAS, F. A., and MOLINILLO, J. M. G. 2010. Structure-activity relationship of benzoxazinones and related compounds with respect to the growth inhibition and α-amylase activity in cress seedlings. *J. Plant Physiol.* 167:1221–1225.

KHANH, T. D., CHUNG, M. I., XUAN, T. D., and TAWATA, S. 2005. The exploitation of crop allelopathy in sustainable agricultural production. *J. Agron. Crop. Sci.* 191:172–184.

KIMURA, M., TAKAHAHI-ANDO, N., NISHIUCHI, T., OHSATO, S., TOKAI, T., OCHIAI, N., FUJIMURA, M., KUDO, T., HAMAMOTO, H., and YAMAGUCHI, I. 2006. Molecular biology and biotechnology for reduction of *Fusarium* mycotoxin contamination. *Pestic. Biochem. Physiol.* 86:117–123.

KNOP, M., PACYNA, S., VOLOSHCHUK, N., KANT, S., MÜLLENBORN, C., STEINER, U., KIRCHMAIR, M., SCHERER, W., and SCHULZ, M. 2007. *Zea mays*: benzoxazolinone detoxification under sulfur deficiency conditions - a complex allelopathic alliance including endophytic *Fusarium verticillioides*. *J. Chem. Ecol.* 33:225–237.

KOBAYASHI, M., SHINOHARA, M., SAKOH, C., KATAOKA, M., and SHIMIZU, S. 1998. Lactone-ring-cleaving enzyme: Genetic analysis, novel RNA editing, and evolutionary implications. *Proc. Natl. Acad. Sci. U. S. A.* 95:12787–12792.

KONG, C. H., WANG, P., GU, Y., XU, X. H., and WANG, M. L. 2008. Fate and impact on microorganisms of rice allelochemicals in paddy soil. *J. Agric. Food Chem.* 56:5043–5049.

KROGH, S. S., MENSZ, S. J. M., NIELSEN, S. T., MORTENSEN, A. G., CHRISTOPHERSEN, C., and FOMSGAARD, I. S. 2006. Fate of benzoxazinone allelochemicals in soil after incorporation of wheat and rye sprouts. *J. Agric. Food Chem.* 54:1064–1074.

KRUIDHOF, H. M., BASTIAANS, L., and KROPFF, M. J. 2008. Ecological weed management by cover cropping: Effects on weed growth in autumn and weed establishment in spring. *Weed Res.* 48:492–502.

KRUIDHOF, H. M., BASTIAANS, L., and KROPFF, M. J. 2009. Cover crop residue management for optimizing weed control. *Plant Soil* 318:169–184.

KRUIDHOF, M., GALLANDT, E. R., HARAMOTO, E. R., and BASTIAANS, L. 2010. Selective weed suppression by cover crop residues: effects of seed mass and timing of species' sensitivity. *Weed Res.* 51:177–186.

La Hovary, C. 2011. Allelochemicals in *Secale cereale*. Biosynthesis and molecular biology of benzoxazinones. Ph.D. dissertation. North Carolina State University.

Liebman, M. and Davis, A. S. 2000. Integration of soil, crop and weed management in low-external-input farming systems. *Weed Res.* 40:27–47.

Liebman, M. and Davis, A. S. 2009. Managing weeds in organic farming systems: an ecological approach, pp. 173–196, *in* C. A. Francis (ed.), Organic Farming: The Ecological System. Agronomy Monograph 54. American Society of Agronomy, Madison, WI, USA.

Liebman, M. and Gallandt, E. R. 1997. Many little hammers: Ecological approaches for management of crop-weed interactions, pp. 291–343, *in* L. E. Jackson (ed.), Ecology in Agriculture. Academic, San Diego, CA, USA.

Liebman, M. and Mohler, C. L. 2001. Weeds and the soil environment, pp. 210–268, *in* M. Liebman, C. L. Mohler, and C. P. Staver (eds.), Ecological Management of Agricultural Weeds. Cambridge University Press, Cambridge, England.

Lundkvist, A., Salomonsson, L., Karlsson, L., and Gustavsson, A.-M. D. 2008. Effects of organic farming on weed flora composition in a long term perspective. *Eur. J. Agron.* 28:570–578.

Macías, F. A., Oliveros-Bastidas, A., Marín, D., Castellano, D., Simonet, A. M., and Molinillo, J. M. G. 2004. Degradation studies on benzoxazinoids. Soil degradation dynamics of 2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one (DIMBOA) and its degradation products, phytotoxic allelochemicals from Gramineae. *J. Agric. Food Chem.* 52:6402–6413.

Macías, F. A., Marín, D., Chinchilla, N., Varela, R. M., Oliveros-Bastidas, A., Marín, D., and Molinillo, J. M. G. 2005a. Structure-activity relationship studies of benzoxazinones, and related compounds. Phytotoxicity on Echinochloa crus-galli (L.) P. Beauv. *J. Agric. Food Chem.* 53:4373–4380.

Macías, F. A., Marín, D., Oliveros-Bastidas, A., Castellano, D., Simonet, A. M., and Molinillo, J. M. G. 2005b. Structure-activity relationships (SAR) studies of benzoxazinones, their degradation products and analogues. Phytotoxicity on standard target species (STS). *J. Agric. Food Chem.* 53:538–548.

Macías, F. A., Oliveros-Bastidas, A., Marín, D., Castellano, D., Simonet, A. M., and Molinillo, J. M. G. 2005c. Degradation studies on benzoxazinoids. Soil degradation dynamics of (2*R*)-2-*O*-β-D-glucopyranosyl-4-hydroxy-(2H)-1,4-benzoxazin-3(4H)-one (DIBOA-Glc) and its degradation products, phytotoxic allelochemicals from Gramineae. *J. Agric. Food Chem.* 53:554–561.

Macías, F. A., Marín, D., Oliveros-Bastidas, A., Castellano, D., Simonet, A. M., and Molinillo, J. M. G. 2006a. Structure-activity relationships (SAR) studies of benzoxazinones, their degradation products and analogues. Phytotoxicity on problematic weeds *Avena fatua* L. and *Lolium rigidum* Gaud. *J. Agric. Food Chem.* 54:1040–1048.

Macías, F. A., Marín, D., Oliveros-Bastidas, A., Chinchilla, D., Simonet, A. M., and Molinillo, J. M. G. 2006b. Isolation and synthesis of allelochemicals from Gramineae: benzoxazinones and related compounds. *J. Agric. Food Chem.* 54:991–1000.

Macías, F.A., Marín, D., Oliveros-Bastidas, A., Simonet A.M., and Molinillo, J.M.G. 2006a. Ecological relevance of the degradation processes of allelochemicals, pp. 91–107, *in* Y. Fujii (ed.), New Concepts and Methodology in Allelopathy. Science Publishers Inc.

Macías, F. A., Molinillo, J. M., Varela, R. M., and Galindo, J. C. 2007. Allelopathy—a natural alternative for weed control. *Pest Manag. Sci.* 63:327–348.

Macías, F. A., Marín, D., Oliveros-Bastidas, A., and Molinillo, J. M. G. 2009. Rediscovering the bioactivity and ecological role of 1,4-benzoxazinones. *Nat. Prod. Rep.* 26:478–489.

Manici, L. M., Caputo, F., and Babini, V. 2004. Effect of green manure on *Pythium* spp. population and microbial communities in intensive cropping systems. *Plant Soil* 263:133–142.

Masiunas, J. B., Weston, L. A., and Weller, S. C. 1995. The impact of rye cover crops on weed populations in a tomato cropping system. *Weed Sci.* 43:318–323.

Matthiessen, J. N. and Kirkegaard, J. A. 2006. Biofumigation and enhanced biodegradation: opportunity and challenge in soilborne pest and disease management. *Crit. Rev. Plant Sci.* 25:235–265.

Mohler, C. L. 1996. Ecological bases for the cultural control of weeds. *J. Prod. Agric.* 9:468–474.

Mwaja, V. N., Masiunas, J. B., and Weston, L. A. 1995. Effects of fertility on biomass, phytotoxicity, and allelochemical content of cereal rye. *J. Chem. Ecol.* 21:81–96.

Nagabhushana, G. G., Worsham, A. D., and Yenish, J. P. 2001. Allelopathic cover crops to reduce herbicide use in sustainable agricultural systems. *Allelopathy J.* 8:133–146.

Nair, M., Whitenack, C. J., and Putnam, A. R. 1990. 2,2′-Oxo-1,1′-azonezene: a microbially transformed allelochemical from 2,3 benzoxazolinone. *J. Chem. Ecol.* 16:353–364.

Narwal, S. S. 1994. pp. 288, Allelopathy in Crop Production. Scientific Publishers, Jodhpur, India.

Narwal, S. S. 2010. Allelopathy in ecological sustainable organic agriculture. *Allelopathy J.* 25:51–72.

Neal, A. L., Ahmad, S., Gordon-Weeks, R., and Ton, J. 2012. Benzoxazinoids in root exudates of maize attract *Pseudomonas putida* to the rhizosphere. *PLoS One* 7(4):e35498. doi:10.1371/journal.pone.0035498.

Niemeyer, H. M. 1988. Hydroxamic Acids (4-Hydroxy-l.4-benzoxazin-3-ones), defense chemicals in the Gramineae. *Phytochemistry* 27:3349–3358.

Niemeyer, H. M. 2009. Hydroxamic acids derived from 2-hydroxy-2h-1,4-benzoxazin-3(4h) one: key defense chemicals of cereals. *J. Agric. Food Chem.* 57:1677–1696.

Niemeyer, H. M. and Jerez, J. M. 1997. Chromosomal location of genes for hydroxamic acid accumulation in *Triticum aestivum* L. (wheat) using wheat aneuploids and wheat substitution lines. *Heredity* 79:10–14.

Niemeyer, H. M., Bravo, H. R., Peña, G. F., and Corcuera, L. J. 1982. Decomposition of 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one, a hydroxamic acid from Gramineae, pp. 22–28, *in* H. Kehl (ed.), Chemistry and Biology of Hydroxamic Acids. Karger, A.G. Basel, Switzerland.

Nomura, T., Ishihara, A., Imaishi, H., Ohkawa, H., Endo, T. R., and Iwamura, H. 2003. Rearrangement of the genes for the biosynthesis benzoxazinones in the evolution of Triticeae species. *Planta* 217:776–782.

Nomura, T., Ishihara, A., Yanagita, R. C., Endo, T. R., and Iwamura, H. 2005. Three genomes differentially contribute to the biosynthesis of benzoxazinones in hexaploid wheat. *Proc. Natl. Acad. Sci. U. S. A.* 102:16490–16495.

Osvald, H. 1953. On antagonism between plants. Proc. 7th Int. Congr. Bot., Stockholm. 167–170.

Perez, F. J. 1990. Allelopathic effect of hydroxamic acids from cereals on *A. sativa* and *A. fatua*. *Phytochemistry* 29:773–776.

Peréz, F. J. and Ormeño-Nuñez, J. 1991. Differences in hydroxamic acid content in root exudates of wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.). Possible role in allelopathy. *J. Chem. Ecol.* 17:1037–1043.

Phillips S. H. and Young Jr., H. M. 1973. No-tillage farming. Reiman Association, Milwaukee WI, 224.

Putnam, A. R. and Defrank, J. 1983. Use of phytotoxic plant residues for selective weed control. *Crop. Prot.* 2:173–181.

Queirolo, C. B., Andreo, C. S., Vallejos, R. H., Niemeyer, H. M., and Corcuera, L. J. 1981. Effects of hydroxamic acids isolated

from graminae on adenosine 5″-triphosphate synthesis in chloroplasts. *Plant Physiol.* 68:941–943.

REBERG-HORTON, S. C., BURTON, J. D., DANEHOWER, D. A., GUOYING, M., MONKS, D. W., MURPHY, J. P., RANELLS, N. N., WILLIAMSON, J. D., and CREAMER, N. G. 2005. Changes over time in the allelochemical content of ten cultivars of rye (*Secale cereale*). *J. Chem. Ecol.* 31:179–192.

RICE, C. P., PARK, Y. B., ADAM, F., ABDUL-BAKI, A. A., and TEASDALE, J. R. 2005. Hydroxamic acid content and toxicity of rye at selected growth stages. *J. Chem. Ecol.* 31:1887–1905.

RICE, C. P., CAI, G., and TEASDALE, J. R. 2012. Concentrations and allelopathic effects of benzoxazinoid compounds in soil treated with rye (*Secale cereale*) cover crop. *J. Agric. Food Chem.* 60:4471–4479.

SANCHEZ-MOREIRAS, A. M. and REIGOSA, M. J. 2005. Whole plant response of lettuce after root exposure to BOA (2(3*H*)-benzoxazoninone). *J. Chem. Ecol.* 31:2689–2703.

SANCHEZ-MOREIRAS, A. M., WEISS, O. A., and REIGOSA-ROGER, M. J. 2003. Allelopathic evidence in the Poaceae. *Bot. Rev.* 69:300–319.

SANCHEZ-MOREIRAS, A. M., OLIVEROS-BASTIDAS, A., and REIGOSA, M. J. 2010. Reduced photosynthetic activity is directly correlated with 2-(3*H*)-benzoxazolinone accumulation in lettuce leaves. *J. Chem. Ecol.* 36:205–209.

SANCHEZ-MOREIRAS, A. M., MARTINEZ-PEÑALVER, A., and REIGOSA, M. J. 2011. Early senescence induced by 2-3*H*-benzoxazolinone (BOA) in *Arabidopsis thaliana*. *J. Plant Physiol.* 168:863–870.

SARRANTONIO, M. and GALLANDT, E. 2003. The role of cover crops in North American cropping systems. *J. Crop. Prod.* 8:53–74.

SAUNDERS, M. and KOHN, L. M. 2008. Host-synthesized secondary compounds influence the *in* V*itro* interactions between fungal endophytes of maize. *Appl. Environ. Microbiol.* 74:136–142.

SCHULLEHNER, K., DICK, R., VITZTHUM, F., SCHWAB, W., BRANDT, W., FREY, F., and GIERL, A. 2008. Benzoxazinoid biosynthesis in dicot plants. *Phytochemistry* 69:2668–2677.

SCHULZ, M. and WEISSENBÖCK, G. 1987. Partial purification and characterization of a luteolin-triglucuronide-specific ß-glucuronidase from rye primary leaves (*Secale cereale*). *Phytochemistry* 26:933–937.

SCHULZ, M. and WEISSENBÖCK, G. 1988. Three specific UDP-glucuronate: flavone-glucuronosyl-transferases from primary leaves of *Secale cereale*. *Phytochemistry* 27:1261–1267.

SCHULZ, M. and WIELAND, I. 1999. Variations in metabolism of BOA among species in various field communities - biochemical evidence for co-evolutionary processes in plant communities? *Chemoecology* 9:133–141.

SCHULZ, M., STRACK, D., WEISSENBÖCK, G., MARKHAM, K. R. G., DELLAMONICA, G., and CHOPIN, J. 1985. Two luteolin 0-glucuronides from primary leaves of *Secale cereale*. *Phytochemistry* 24:343–345.

SCHULZ, M., KANT, S., KNOP, M., SICKER, D., COLBY, T., HARZEN, A., and SCHMIDT, J. 2008. The allelochemical benzoxazolinone—molecular backgrounds of its detoxification and degradation. 5th World Congress on Allelopathy, p. 63, Saratoga Springs, NY, USA, 21–25. September 2008.

SCHULZ, M., POURMOYYED, P., WAGNER, S., FREY, M., GIERL, A., DRESEN-SCHOLZ, B. 2011. Benzoxazinone detoxification in transgenic *Arabidopsis thaliana*. 6th World Congress on Allelopathy, p. 22, Guanghzou, China. 15–19 December 2011.

SCHULZ, M., SICKER, D., BALUŠKA, F., SABLOFSKI, T., SCHERER, H. W., and RITTER, F. M. 2012a. Benzoxazolinone detoxification and degradation—A molecule´s journey, pp. 17–42, *in* M. N. Abd El-Ghany Hasaneen (ed.), Herbicides—Properties, Synthesis and Control of Weeds. InTech, Rijeka, Croatia.

SCHULZ, M., MAROCCO, A., and TABAGLIO, V. 2012b. BOA detoxification of four summer weeds during germination and seedling growth. *J. Chem. Ecol.* 38:933–946.

SHILLING, D. G., LIEBL, R. A., and WORSHAM, A. D. 1985. Rye (*Secale cereale* L.) and wheat (*Triticum aestivum* L.) mulch: The suppression of certain broad-leaved weeds and the isolation and identification of phytotoxins, pp. 243–271, *in* A. C. Thompson (ed.), The Chemistry of Allelopathy: Biochemical Interactions Among Plants. American Chemical Society, Washington, DC, USA.

SICKER, D. and SCHULZ, M. 2002. Benzoxazinones in plants: Occurrence, synthetic access, and biological activity. *Stud. Nat. Prod. Chem.* 27:185–232.

SICKER, D., FREY, M., SCHULZ, M., and GIERL, A. 2000. Role of natural benzoxazinones in the survival strategies of plants, pp. 319–346, *in* K. W. Jeong (ed.), International Review of Cytology —A Survey of Cell Biology Vol. 198. Academic, San Diego, California.

SICKER, D., SCHNEIDER, B., HENNIG, L., KNOP, M., and SCHULZ, M. 2001. Glucoside carbamate from benzoxazolin-2(3*H*)-one detoxification in extracts and exudates of corn roots. *Phytochemistry* 58:819–825.

SICKER, D., HAO, H., and SCHULZ, M. 2003. Benzoxazolin-2-(*3H*)-ones. Generation, effects and detoxification in the competition among plants, pp. 77–102, *in* F. A. Macias, J. C. G. Galindo, J. M. G. Molinillo, and H. G. Cutler (eds.), Recent Advances on Allelopathy Vol. II. CRC Press, Boca Raton (FL).

SINGH, H. P., BATISH, D. R., KAUR, S., SETIA, N., and KOHLI, R. K. 2005. Effects of 2-benzoxazolinone on the germination, early growth and morphogenetic response of mung bean (*Phaseolus aureus*). *Ann. Appl. Biol.* 147:267–274.

SMEDA, R. J. and WELLER, S. C. 1996. Potential of rye (*Secale cereale*) for weed management in transplant tomatoes (*Lycopersicon esculentum*). *Weed Sci.* 44:596–602.

STRACK, D., MEURER, B., and WEISSENBÖCK, G. 1982. Tissue-specific kinetics of flavonoid accumulation in primary leaves of rye (*Secale cereale* L.). *Z. Pflanzenphysiol.* 108:131–141.

STRACK, D., ENGEL, U., WEISSENBÖCK, G., GROTJAHN, L., and WRAY, V. 1986. Ferulic acid esters of sugar carboxylic acids from primary leaves of rye (*Secale cereale*). *Phytochemistry* 25:260–2608.

SUE, M., NAKAMURA, C., and NOMURA, T. 2011. Dispersed benzoxazinone gene cluster: molecular characterization and chromosomal localization of glucosyltransferase and glucosidase genes in wheat and rye. *Plant Physiol.* 157:985–997.

TABAGLIO, V., GAVAZZI, C., SCHULZ, M., and MAROCCO, A. 2008. Alternative weed control using the allelopathic effect of natural benzoxazinoids from rye mulch. *Agron. Sustain. Dev.* 28:397–401.

TEASDALE, J. R. and MOHLER, C. L. 1993. Light transmittance, soil temperature, and soil moisture under residue of hairy vetch and rye. *Agron. J.* 85:673–680.

TEASDALE, J. R. and MOHLER, C. L. 2000. The quantitative relationship between weed emergence and the physical properties of mulches. *Weed Sci.* 48:385–392.

TEASDALE, J. R., MANGUM, R. W., RADHAKRISHNAN, J., and CAVIGELLI, M. A. 2004. Weed seedbank dynamics in three organic farming crop rotations. *Agron. J.* 96:1429–1435.

TEASDALE, J. R., RICE, C. P., CAI, G. C., and MANGUM, R. W. 2012. Expression of allelopathy in the soil environment: soil concentration and activity of benzoxazinoid compounds released by rye cover crop residue. *Plant Ecol.*. doi:10.1007/s11258-012-0057-x.

TET-VUN, C. and ISMAIL, B. S. 2006. Field evidence of the allelopathic properties of *Dicranopteris linearis*. *Weed Biol. Manag.* 6:59–67.

THARAYIL, N. 2009. To survive or to slay: resource-foraging role of metabolites implicated in allelopathy. *Plant Signal. Behav.* 4:580–583.

UNDERSTRUP, A. G., RAVNSKOV, S., HANSEN, H. C. B., and FOMSGAARD, I. S. 2005. Biotransformation of 2-Benzoxazolinone intro 2-

amino-(3*H*)-phenoxazin-3-one and 2-acetylamino-(3*H*)-Phenoxazin-3-one in soil. *J. Chem. Ecol.* 31:1205–1222.

VARELA, E. and TIEN, M. 2003. Effect of pH and oxalate on hydroquinone-derived hydroxyl radical formation during brown rot wood degradation. *Appl. Environ. Microbiol.* 69:6025–6031.

VIRTANEN, A. I. and HIETALA, P. K. 1960. Precursors of benzoxazolinone in rye plants. I. Precursor II, the aglucone. *Acta Chem. Scand.* 14:499–502.

VLYSSIDES, A., BARAMPOUTI, E. M., MAI, S., SOTIRIA, M., and ELENI, N. 2011. Degradation and mineralization of gallic acid using Fenton's Reagents. *Environ. Eng. Sci.* 28:515–520.

VON RAD, U., HÜTTL, R., LOTTSPEICH, F., GIERL, A., and FREY, M. 2001. Two glucosyltransferases involved in detoxification of benzoxazinoids in maize. *Plant J.* 28:633–642.

WALLACE, J. 2001 (ed.). Organic Field Crop Handbook, p. 292. Canadian Organic Growers, 2nd ed., Ottawa, Ontario, Canada.

WALTERS, S. A. and YOUNG, B. G. 2008. Utility of winter rye living mulch for weed management in zucchini squash production. *Weed Technol.* 22:724–728.

WEIDENHAMER, J. D., MORTON, T. C., and ROMEO, J. T. 1987. Solution volume and seed number: Often overlooked factors in allelopathic bioassays. *J. Chem. Ecol.* 13:1481–1491.

WEIH, M., DIDON, U. M. E., RÖNNBERG-WÄSTLJUNG, A.-C., and BJÖRKMAN, C. 2008. Integrated agricultural research and crop breeding: Allelopathic weed control in cereals and long-term productivity in perennial biomass crops. *Agric. Syst.* 97:99–107.

WESTON, L. A. 1996. Utilization of allelopathy for weed management in agroecosystems. *Agron. J.* 88:860–866.

WESTON, L. A. and DUKE, S. O. 2003. Weed and Crop Allelopathy. *Crit. Rev. Plant Sci.* 22:367–389.

WESTON, L., ALSAADAW, I., and BAERSON, S. 2013. Sorghum allelopathy – From ecosystem to molecule. *J. Chem. Ecol*, 39:142–153.

WIELAND, I., KLUGE, M., SCHNEIDER, B., SCHMIDT, J., SICKER, D., and SCHULZ, M. 1998. 3-ß-D-Glucopyranosyl-benzoxazolin-2 (3*H*)-one - a detoxification product of benzoxazolinone in oat roots. *Phytochemistry* 49:719–722.

WIELAND, I., FRIEBE, A., KLUGE, M., SICKER, D., and SCHULZ, M. 1999. Detoxification of 2-(3H)-benzoxazolinone in higher plants, pp. 47–56, *in* F.A. Macias, J.C.G. Galindo, J.M.G. Molinillo and H.G. Cutler (eds.), Recent Advances in Allelopathy. A Science for the Future. Universidad Cadiz (E).

WOJCIK-WOJTKOWIAK, D., POLITYCKA, B., SCHNEIDER, I. M., and PERKOWSKI, J. 1990. Phenolic substances as allelopathic agents arising during the degradation of rye *(Secale cereale)* tissues. *Plant Soil* 124:143–147.

WOODWARD, M. D., CORCUERA, L. J., HELGESON, J. P., and UPPER, C. D. 1978. Decomposition of 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one in aqueous solution. *Plant Physiol.* 61:796–802.

WORSHAM, A. D. 1991. Allelopathic cover crops to reduce herbicide inputs. *Proc. South. Weed Sci. Soc.* 44:58–69.

WORTHINGTON, M., and REBERG-HORTON, C. 2013. Breeding cereal crops for enhanced weed suppression: Optimizing allelopathy and competitive ability. *J. Chem. Ecol.*, 39:213–231.

WU, H., PRATLEY, J., MA, W., and HAIG, T. 2003. Quantitative trait loci and molecular markers associated with wheat allelopathy. *Theor. Appl. Genet.* 107:1477–1481.

YUE, Q., BACON, C., and RICHARDSON, M. 1998. Biotransformation of 2-benzoxazolinone and 6-methoxybenzoxazolinone by *Fusarium moniliforme*. *Phytochemistry* 48:451–454.

ZHANG, Z.-Y., PAN, L.-P., and LI, H.-H. 2010. Isolation, identification and characterization of soil microbes which degrade phenolic allelochemicals. *J. Appl. Microbiol.* 108:1839–1849.

ZIKMUNDOVA, M., DRANDAROV, K., BIGLER, L., HESSE, M., and WERNER, C. 2002a. Biotransformation of 2-benzoxazolinone and 2-hydroxy-1,4-benzoxazin-3-one by endophytic fungi isolated from *Aphelandra tetragona*. *Appl. Environ. Microb.* 68:4863–4870.

ZIKMUNDOVA, M., DRANDAROV, K., HESSE, M., and WERNER, C. 2002b. Hydroxylated 2-amino-3H-phenoxazin-3-one derivatives as products of 2-hydroxy-1,4-benzoxazin-3-one (HBOA) biotransformation by *Chaetosphaeria* sp., an endophytic fungus from *Aphelandra tetragona*. *Z. Naturforsch. C* 57C. doi:660.