

Allelopathic cover crop of rye for integrated weed control in sustainable agroecosystems

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Abstract

The allelopathic potential of rye (*Secale cereale* L.) is mainly due to phytotoxic benzoxazinones, compounds that are produced and accumulated in young tissues to different degrees depending on cultivar and environmental influences. Living rye plants exude low levels of benzoxazinones, while cover crop residues can release from 12 to 20 kg ha⁻¹. This paper summarizes the results obtained from several experiments performed in both controlled and field environments, in which rye was used as a cover crop to control summer weeds in a following maize crop. Significant differences in benzoxazinoid content were detected between rye cultivars. In controlled environments, rye mulches significantly reduced germination of some broadleaf weeds. Germination and seedling growth of *Amaranthus retroflexus* and *Portulaca oleracea* were particularly affected by the application of rye mulches, while *Chenopodium album* was hardly influenced and *Abutilon theophrasti* was advantaged by the presence of the mulch. With reference to the influence of agronomic factors on the production of benzoxazinoids, nitrogen fertilization increased the content of allelochemicals, although proportionally less than dry matter. The field trial established on no-till maize confirmed the significant weed suppressiveness of rye mulch, both for grass and broadleaf weeds. A significant positive interaction between N fertilization and no-tillage resulting in the suppression of broadleaf weeds was observed. The different behavior of the weeds in the presence of allelochemicals was

explained in terms of differential uptake and translocation capabilities. The four summer weeds tested were able to grow in the presence of low amounts of benzoxazolin-2(3H)-one (BOA), between 0.3 and 20 µmol g⁻¹ fresh weight. Although there were considerable differences in their sensitivity to higher BOA concentrations, *P. oleracea*, *A. retroflexus*, and *Ch. album* represented a group of species with a consistent absorption capability. The insensitivity of *A. theophrasti* to BOA was due to reduced accumulation in seedlings. Overall, results confirm that the use of a rye cover crop in a suitable crop rotation represents a sustainable weed management practice permitting a reduction in the amount of herbicides used in agroecosystems, thus limiting the environmental risks of intensive agriculture.

Introduction

In conventional agriculture the herbicidal control of weeds has raised concern regarding its effect on the quality of soil, water, animal and human health, and food (Doran and Safley, 1997; Gavazzi *et al.*, 2010). Thus it has become necessary to reduce the use of synthetic herbicides without lowering farm profitability. To accomplish significant reductions in herbicide use, a strategy based on both chemical refinement and integrated crop management has been advocated (Mortensen *et al.*, 2000). Herbicides remain the central tool of crop protection, but new solutions have to be adopted, such as improved application technology and timing, factor-adjusted dosages, precision agriculture techniques and the development of herbicides with low environmental impact. In addition to these curative tactics, integrated weed management should be seen as a component of a sustainable agroecosystem. Sustainable weed management involves numerous practices, such as crop rotation, cover crops, cultivations, weeding, flaming, biocontrol, weed seed predation, smoother crops, competition, mulching, natural herbicides and allelopathy (Wallace, 2001; Flamini, 2012; Schulz *et al.*, 2013). All these mechanisms can contribute to controlling the weeds in conventional or organic farming systems (Bàrberi, 2002; Kalinova, 2010) and reducing the need for herbicides.

Cover cropping fits very well in such an integrated approach, as it provides many additional services for the agroecosystem, including the improvement of soil structure and water infiltration, the reduction of soil erosion, an increase in soil fertility and nutrient cycling, a reduction in soil nutrient losses due to leaching, the enhancement of biodiversity, and a contribution to weed and pest management (Altieri, 1987; Sarrantonio and Gallandt, 2003; Davis, 2010). Cover cropping involves actively growing non-harvested crops. The weed control effect operates both during the growth of the cover crop and after its termination with the formation of mulch. Weed suppression by mulch is attributed to various physical and chemical factors. Physical effects include shading and lower soil temperature. The chemical effects

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include toxic microbial products and pH changes in the soil, and allelopathy (Clark, 2007); the latter is due to the fact that some cover crops also produce relevant amounts of allelochemicals, which are released from living or dead plant tissues and can exert a strong influence on weed germination and growth (Liebman and Davis, 2009). The degree of phytotoxicity depends on the type of residue (concentration and content of allelochemicals), maturity stage of the cover, and extent of weathering (Burgos *et al.*, 1999; Inderjit and Keating, 1999; Bàrberi and Mazzoncini, 2001; Teasdale *et al.*, 2012).

In plants, allelochemicals are bioactive secondary metabolites such as glucosinolates, phenolic compounds, terpenoids, alkaloids, hydroxamic acids, etc., distributed broadly through plant organs (Haig, 2008). The characteristic secondary compounds of several Poaceae are benzoxazinone- β -D-glucosides (Schulz *et al.*, 2012). The compounds are stored in the vacuole until the tissue is damaged and hydrolysis of the sugar moiety by β -glucosidases takes place. The highly bioactive aglycones can also be released into the soil by root exudation or by plant residue degradation (Barnes and Putnam, 1987). After deglucosylation, the aglycones undergo a ring contraction, yielding benzoxazolinones. For this reason, benzoxazolinones have a short lifetime whereas benzoxazolinones persist in the soil for several weeks. The most important allelopathic secondary products in rye (*Secale cereale* L.) are glucosylated benzoxazinones, 2,4-dihydroxy-2H-1,4-benzoxazin-3(4H)-one (DIBOA glucoside) in the shoots and 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (DIMBOA glucoside) in the roots (Copaja *et al.*, 2006). They occur together with the aglycones and their degradation products: two benzoxazolinones, MBOA (6-methoxy-benzoxazolin-2(3H)-one and BOA (Barnes *et al.*, 1987). The concentrations of BOA and DIBOA depend on rye genotype, plant organ, plant age, on the fertility regime, and on environmental factors: temperature, water supply, photoperiod, UV irradiation, and light intensity (Niemeyer, 2009). The biosynthesis of the compounds has been investigated in maize (Frey *et al.*, 2009; 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 glucosyltransferases. The product DIBOA-glucoside is the precursor of DIMBOA-glucoside. The final steps of DIMBOA-glucoside synthesis are catalyzed by dioxygenase BX6 and methyltransferase BX7.

Rye is one of the best cool-season cover crops and is widely used for its high biomass production, earliness, wide soil and climate adaptability, and exceptional weed suppression potential (Batish *et al.*, 2001; Clark, 2007; Tabaglio *et al.*, 2008; Gavazzi *et al.*, 2010). Rye effectively suppresses weeds by shading, competition and allelopathy. The suppression can occur while the rye crop is living, due to its thick and tall stand. At termination of the cover crop, chemicals are released from the dead mulch produced by mowing, chopping, rolling or spraying the biomass. The mulch can be left on the soil surface, or tilled in by disking or plowing. The level of weed suppression depends on the thickness of the mulch layer, with an exponential relationship between mulch mass and weed inhibition (Teasdale and Mohler, 2000). The rate and the time course of allelochemical release from rye mulch are largely dependent on the amount and decomposition of the residue, on soil and environmental conditions and on residue management. Crop residues left on the soil surface decompose more slowly than residues incorporated into the soil, which may result in a slower but longer-lasting release rate of allelochemicals (Kruidhof *et al.*, 2009). When the residue material is retained on the soil surface, the effective weed control can be observed for 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 chopped prior to its incorporation into the soil. This treatment may influence the decomposition rate and thereafter the release of allelochemicals (Angers and Recous, 1997).

The objectives of the present work were: i) to verify the extent of variability of the benzoxazinoid contents in different rye genotypes; ii) to test the effect of nitrogen fertilization levels on benzoxazinoid contents in four rye cultivars grown in a greenhouse; iii) to verify the weed suppression ability of the mulches under conventional and no-tillage systems and three levels of nitrogen fertilization in field conditions; iv) to study how the weeds differ in their BOA uptake ability during germination and the early stage of growth.

Materials and methods

Plant material

Eight cultivars (Born, Fasto, Forestier, Matador, Nikita, Primizia, Protector and Treviso) of rye (*Secale cereale* L.) were used in experiment A, whereas a selection of four cultivars (Fasto, Forestier, Nikita, and Primizia) were included in experiment B. The field trial was set up using cv. Primizia as the cover crop (experiment C). Four summer weeds (velvetleaf *Abutilon theophrasti* Medicus, redroot pigweed *Amaranthus retroflexus* L., common lambsquarters *Chenopodium album* L., common purslane *Portulaca oleracea* L.) were used for experiments A and D, while only the last three weeds were tested in experiment B. In experiment C only the weeds which emerged from the natural seed bank were counted in plots planted with the maize hybrid PR34N43 (Pioneer Hi-Bred Italia, Cremona, Italy).

Determination of benzoxazinoids

For DIBOA and BOA determination, samples of rye dry shoots were ground to pass through a 40-mesh screen in a Wiley mill. Ground tissues were stored in amber-coloured bottles at room temperature until the extraction of allelochemicals. The dried plant material was thoroughly mixed to obtain a homogenous sample. Aliquots of 80 mg were extracted with 50% methanol (1:45 w/v). The mixture was mortared for further homogenization for 15 min and centrifuged (15 min at 10,000 g). The supernatant was used for high-performance liquid chromatography (HPLC) (Beckman Coulter, Brea, CA, USA, pump module 126 equipped with a diode array detector 166). For DIBOA and DIBOA-glucose determinations the following gradient was used: 0-10 min 100% A (H_2O , 0.1% TFA); 10-30 min 25% A in B (methanol); 30-35 min 100% B. Compounds were separated with an analytical Nucleodur 100-5 C18 column (Macherey and Nagel, Dueren, Germany); the detection wavelengths were 280 and 227 nm. DIBOA and DIBOA-glucose were calculated in sum using external standard curves for quantifying. The data obtained were from three different extractions. BOA determinations were performed using the isocratic system described by Reberg-Horton *et al.* (2005) and with the following gradients: (gradient A): 0-1 min 100% A (H_2O , 0.1% TFA), 1-8 min 45% A in B (methanol), 8-16 min 32% A in B; 16-19 min 100% B. Gradient B: 0-1 min 100% A, 1-8 min 60% A in B, 8-20 min 32% A in B, 20-22 min 100% B. BOA determinations were run with an analytical ultrasphere ODS RP 18 column (Beckman Coulter); the detection wavelengths were 280 and 227 nm. Calculations of content were based on external standard curves. The data obtained were from six different extractions. The reference compounds DIBOA and DIBOA-glucose were donated by Prof. Dieter Sicker, University of Leipzig, Germany. The BOA was purchased from Fluka-Sigma-Aldrich (St. Louis, MO, USA).

Experiment A: variability of the benzoxazinoid contents in different rye genotypes and inhibitory effect of mulches on summer weeds

The first study was carried out in an unheated greenhouse and

involved eight rye cultivars. The randomized block design had 8 cultivars and 6 replications. Fifty-four plastic pots (59.5×18×14 cm, with a surface area of 0.11 m²) were used, each containing 12 kg of dry loam soil. On December 15, 2004, 40 germinable rye kernels were seeded in each pot. The plants were grown in the open to better approach field conditions, under unlimited water availability, and were not fertilized. Each cultivar was cut at the heading stage. Shoot tissues were cut at ground level, oven-dried for 3 days at 60°C, and weighed (Burgos *et al.*, 1999). The dry shoots were cut into 1-cm lengths. BOA and DIBOA contents were determined as reported above.

Rye biomass was used as mulch to evaluate its inhibitory effect on four summer weeds grown in the greenhouse. The rye biomass was redistributed over the pots, and control pots without mulch were included in the test. On June 13, 2005, 60 viable seeds were sown in each pot for each weed, arranged in a randomized block design with three replications. Seedlings which had emerged were counted at cotyledon appearance and removed up to 32 days after sowing.

Experiment B: effect of nitrogen fertilization on benzoxazinoid contents

Following the first screening, the second experiment aimed to examine the DIBOA and DIBOA-Glucose content in the four rye cultivars with the highest amount of allelochemicals, as influenced by the N fertilization rate (N_0 =control, no fertilization; N_{50} =50 kg N ha⁻¹). The treatments (Cultivars×N rates) were replicated eight times in a split-plot design. The rye was sown on November 23, 2005, with 40 viable kernels per pot. The pots were kept in the open to better approach field conditions. Each cultivar was harvested at heading stage. DIBOA and DIBOA-Glucose determination on rye biomass was conducted with the methodology already described. The experiment was continued with mulch formation on a new pot series. The experimental treatments comprised three factors: i) mulch managements: mulch *vs* no-mulch, ii) four rye cultivars, iii) N fertilization rates: 0 and 50 kg N ha⁻¹. The treatments were replicated four times in a split-split-plot design. On May 19, 2006, 60 viable seeds of three summer weeds (redroot pigweed, common lambsquarters, and common purslane) were sown per pot. The emerged seedlings were counted at cotyledon appearance and removed up to 32 days after sowing.

Experiment C: tillage systems, N fertilization rates and weed suppression ability of the mulches in field conditions

The rye cover crop was sown in the no-till plots of a comparison trial between no-till *vs.* conventional tillage under three N fertilization rates (0, 250 and 300 kg N ha⁻¹) that had been in progress since 2004 at Terranova de' Passerini (Lodi, Northern Italy) on a silt loam (Tabaglio and Gavazzi, 2009). The experimental design was a split-plot with four replications, in which the main factor was Soil Tillage System and the secondary one was N Fertilization Rate. The rye cover crop (cv. Primizia) was sown on November 3, 2006, at 150 kg seeds ha⁻¹ in the no-tillage plots. The rye crop was not fertilized directly, but it used the residual nitrogen from previous fertilizer applications. The rye cover crop was terminated on April 17, 2007, and maize was sown on April 21, 2007, in conventional and no-tillage plots. The rye biomass was weighed, chopped and left on the soil surface as mulch. Plant samples were taken for DIBOA and DIBOA-Glucose determination with the usual methodology. The weed seedlings were counted 19 days after maize sowing (corresponding to 23 days after mulching), from 35×2 cm² diameter area in each plot.

Experiment D: benzoxazolin-2(3H)-one treatments and benzoxazolin-2(3H)-one uptake in weed seedlings

Seeds of four summer weeds were aeroponically grown on cheese-

cloth at 30°C under greenhouse conditions. After 10 d, the seedlings were harvested and incubated on Petri dishes for 24 h with (0.1 mM) 4, (0.5 mM) 20, (1 mM) 40, (2 mM) 80, (5 mM) 200 µmol BOA g⁻¹ fresh weight (FW). Prior to the study, the BOA concentrations used for the germination tests and seedling growth experiments had been evaluated for each species to cover the BOA concentrations where the species exhibits no inhibition or pronounced inhibition. After incubation, the seedlings were washed with water, carefully dried, and separated into roots and shoots. Plant material was extracted immediately with 70% methanol (3 mL g⁻¹ FW) by mortaring with sea sand. Homogenates were centrifuged for 15 min at 14,000 g, and supernatants were used for HPLC analysis. HPLC was performed as described above. All the experiments were repeated independently at least three times.

Statistical analysis

Analysis of variance (ANOVA) was carried out for statistical analysis of all data (MSTAT-C Software, MSU, East Lansing, MI, USA), and Duncan's or Tukey's test ($P<0.05$) was used for mean separation.

Results and discussion

The average content of the benzoxazinoids 2,4-dihydroxy-1,4 (2H)-benzoxazin-3-one (DIBOA) and benzoxazolin-2(3H)-one (BOA) is presented in Table 1. The DIBOA and BOA content of rye mulch was statistically different among cultivars ($P\leq 0.01$), ranging from 177 (cv. Born) to 545 µg g⁻¹ (cv. Fasto). These results demonstrate that high levels of variability in allelochemical production exist, up to three times as much being produced in the best accumulating cultivars.

To measure the allelopathic effect of rye residues on weeds, pots were prepared with the mulch from all eight cultivars. The numbers of weed seedlings emerging from the mulch at 32 days after planting are presented in Table 2. The mulches did not significantly affect emergence of *A. theophrasti* and *Ch. album* seedlings, while for *A. retroflexus* and *P. oleracea* significant effects were detected, although at different levels of probability (from 1% to 100%). As regards *A. theophrasti*, as found in a previous study (Tabaglio and Gavazzi, 2006), rye mulch tends to promote germination, probably because the mulch determines favourable humidity and temperature conditions in the soil. For *A. retroflexus*, all rye mulches, with the exception of Treviso, differ from the control: the greatest weed suppression occurred with

Table 1. Content of benzoxazinoids (DIBOA+BOA) in eight rye cultivars pot-grown in the greenhouse.

Cultivars	DIBOA+BOA (µg g ⁻¹ DM)
Born	177 ^a
Fasto	545 ^c
Forestier	400 ^b
Matador	329 ^{ab}
Nikita	287 ^{ab}
Primizia	397 ^b
Protector	225 ^a
Treviso	266 ^{ab}
Significance	0.01

Within the column, means followed by the same letter are not significantly different at $P\leq 0.05$ according to Duncan's test. DIBOA, 2,4-dihydroxy-1,4 (2H)-benzoxazin-3-one; BOA, benzoxazolin-2(3H)-one; DM, dry matter.

the mulch of the cv. Matador (–52% of *A. retroflexus* seedlings compared with the control), while at the other extreme was Treviso (–19%). For *P. oleracea*, all the mulches were effective: the greatest weed suppression was achieved with Protector (–74%), while the least effective was Forestier (–40%). The results confirm that the larger-seeded species *A. theophrasti* is less sensitive to allelochemicals (Chase *et al.*, 1991) and that seed mass is particularly important for the selective suppression of weeds with crop residues (Mohler, 1996; Liebman and Davis, 2000). The failure to suppress *Chenopodium album* may indicate that this species possesses either highly active mechanisms that avoid the uptake of BOA or efficient detoxification activities in seedlings.

In a subsequent experiment, the effect of nitrogen fertilization on the content of benzoxazinoids was investigated for four cultivars (Fasto, Forestier, Primizia and Nikita), selected on the basis of their DIBOA and BOA content. The rye plants were grown in pots kept in the open to better approach field conditions. Each cultivar was harvested at heading stage. The average content of benzoxazinoids was significantly higher ($P < 0.01$) at the fertilization rate of 50 kg N ha⁻¹ compared to the N₀ level (258 vs 154 µg g⁻¹ of dry matter, i.e. +68%). The interaction rye cultivars×N fertilization rates was not statistically significant for benzoxazinoid content. Significant interaction was obtained in a field experiment, where cv. Primizia was used as the rye cover crop and fertilized with three rates of nitrogen (N₀, N₂₅₀ and N₃₀₀). The data demonstrated that the effects of 250 and 300 kg N ha⁻¹ increased the rye biomass from 81% to 135% compared to the N₀. The total benzoxazinoids applied to soil as rye mulch were higher in the N₂₅₀ and N₃₀₀ plots, with increases of 57% and 105%, respectively over the unfertilized plots (Gavazzi *et al.*, 2010). Therefore, nitrogen fertilization was found to increase the content of allelochemicals, although proportionally less than the increase in dry matter. However, other authors have found higher benzoxazinoid levels under the N₀ treatment (Mwaja *et al.*, 1995; Reberg-Horton *et al.*, 2005).

Table 3 shows the effect of rye mulch (cv. Primizia) on the weed population in a maize crop under conventional tillage and no-till regimes. The rye mulch left on the no-tillage plots significantly reduced the emergence of both grass and broadleaf weeds, although at different probability levels. Grass weeds in no-tillage plots were reduced by 61% compared with those in conventional tillage plots, whereas broadleaf weeds were reduced by 96%. 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. Many other authors have confirmed a lower sensibility in grass weeds, but with very different values (Norsworthy, 2003; Tet-Vun and Ismail, 2006). Overall, in no-tillage plots 783 weed seedlings m⁻² were counted, compared with 2313 in conventional tillage plots. This suppressive effect on weeds, although partial, should be exploited as part of an integrated weed control strategy, because it can permit reduction in amounts of herbicide applied to the maize crop following the rye cover crop. As regards the interaction between the tillage system and N fertilization, a significant effect was found only for broadleaf weeds, which were reduced by more than 80% in the fertilized no-tillage plots (Figure 1). On the contrary, under conventional tillage the broadleaf weeds increased by between 60% and 65%, depending on the fertilization level.

To elucidate the different reactions to rye mulch, four warm-season weeds, *A. theophrasti*, *A. retroflexus*, *Ch. album*, and *P. oleracea*, were tested for their dynamics of BOA uptake in roots and translocation in shoots. Accumulation of BOA in roots increased with increasing BOA concentrations in solution for all species (Figure 2). BOA increased drastically in the roots of *P. oleracea*, *Ch. album*, and *A. retroflexus*, while in *A. theophrasti* the accumulation was lower. The correlation

Table 2. Percentage of weed seedlings which emerged up to 32 days after sowing of four weeds, compared to the control without mulch. Analysis was performed using real data.

Cultivars	Emerged weed seedlings (% of the control without mulch)			
	<i>Abutilon theophrasti</i>	<i>Amaranthus retroflexus</i>	<i>Chenopodium album</i>	<i>Portulaca oleracea</i>
Born	118	55 ^a	107	47 ^{ac}
Fasto	106	54 ^a	103	48 ^{ac}
Forestier	125	60 ^{ab}	104	60 ^{bc}
Matador	142	48 ^a	101	36 ^{ab}
Nikita	120	54 ^a	98	30 ^a
Primizia	123	52 ^a	89	34 ^{ab}
Protector	120	60 ^{ab}	84	26 ^a
Treviso	106	81 ^{bc}	92	48 ^{ac}
Control	100	100 ^c	100	100 ^d
Significance	n.s.	0.01	n.s.	0.001

Within the same column, means followed by the same letter are not significantly different at $P \leq 0.05$ according to Duncan's test. n.s., not significant.

Table 3. Effects of rye mulch on weed population emerged at 23 days after mulching in the following maize crop. Statistical analysis performed using square-rooted data.

Tillage systems	Grass weeds	Broadleaf weeds	Total weeds
Conventional tillage (ploughing)	1999 ^b	313 ^b	2313 ^b
No-tillage	771 ^a	12 ^a	783 ^a
Significance	0.10	0.05	0.10

Within the same column, means followed by the same letter are not significantly different at $P \leq 0.10$ (grass weeds) or $P \leq 0.05$ (broadleaf weeds) according to Tukey's test.

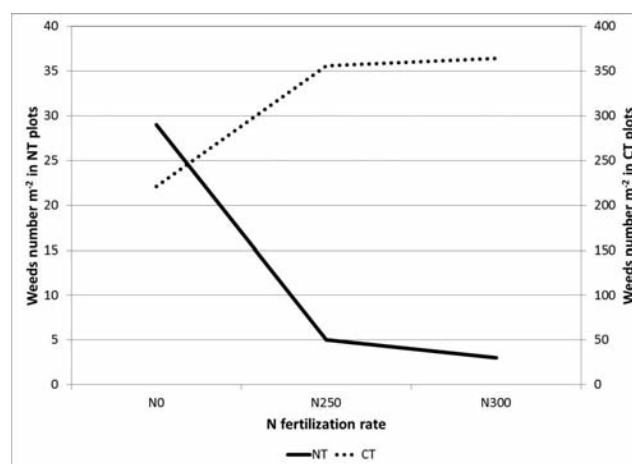


Figure 1. The effect of interaction tillage system×N fertilization on broadleaf weed seedlings, expressed as number of weeds per 1 m² sample area, at 23 days after mulching.

between BOA concentration in solution and in root tissues was significant for all the species. Regression coefficients were ≥ 0.92 for *A. retroflexus*, *Ch. album*, and *P. oleracea*, and decreased to 0.77 for *A. theophrasti*. Two patterns of uptake were evident: *A. theophrasti* and *A. retroflexus* were characterized by a low slope ranging from 9.6 to 12.5 nmol BOA g⁻¹ FW/ μ mol BOA g⁻¹ FW; *Ch. album* and *P. oleracea* showed higher uptakes with slope values approaching 32 nmol BOA g⁻¹ FW/ μ mol BOA g⁻¹ FW.

The concentrations of BOA in the shoots of the four weeds are shown in Figure 3. As reported for roots, the BOA concentrations increased with the BOA concentration in all weeds. Translocation into *A. theophrasti* shoots was low compared to the other weeds. This could be one explanation for allelopathic inefficacy in controlling this weed. *Ch. album* started to translocate BOA the most effectively, resulting in 2- to 3-fold accumulation of the compound in the shoots compared to the roots when incubated with increasing BOA concentrations. Regression coefficients were high, as in roots, for *A. retroflexus*, *Ch. album*, and *P. oleracea*, with the exception of the lower R² for *A. theophrasti* (0.54). The best function for *Ch. album* was represented by a logarithmic equation with R²=0.83.

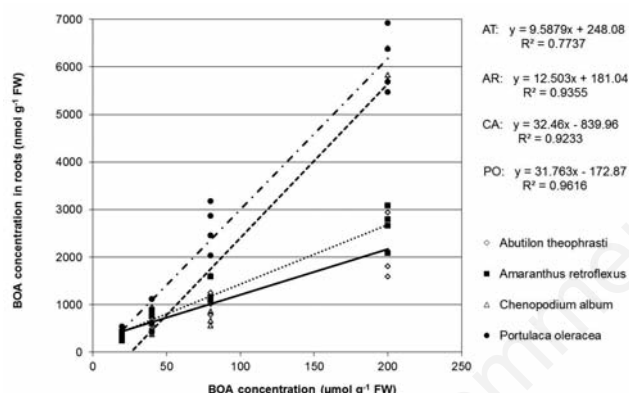


Figure 2. Regression equations between benzoxazolin-2(3H)-one (BOA) concentration in solution and free BOA concentration in the roots of four weed seedlings.

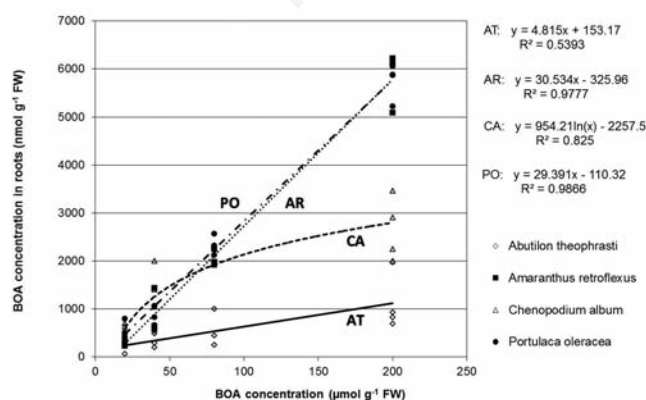


Figure 3. Regression equations between benzoxazolin-2(3H)-one (BOA) concentration in solution and free BOA concentration in the shoots of four weed seedlings.

Conclusions

These works demonstrate that there are significant differences between the rye cultivars grown as regards their benzoxazinoid content. In controlled environments, rye mulches significantly affect the germination of some broadleaf weeds, although no correlation has been found between total benzoxazinoid content and the number of weed seedlings suppressed, suggesting that benzoxazinoids are not the only source of phytotoxicity. Germination and seedling growth of *A. retroflexus* and *P. oleracea* are specifically affected by the application of rye mulches. On the contrary, *A. theophrasti* seems to be advantaged as regards germination by the presence of a layer of mulch. The rate of nitrogen fertilization applied to the rye cover crop influences results: the benzoxazinoid content is significantly higher at a rate of 50 kg N ha⁻¹ than without nitrogen application. The field trial on no-till maize confirms a significant weed suppressiveness of rye mulch, both for grass and broadleaf weeds. As regards the broadleaf weeds, there is a significant interaction between the tillage system and N fertilization: in no-tillage, nitrogen application much reduces the weeds due to the presence of mulch, while under conventional tillage it causes an increase in weed populations.

The different behavior of the weeds with respect to allelochemicals is explained in terms of differential uptake and translocation capabilities. The four summer weeds tested are able to grow in the presence of low amounts of BOA, between 0.3 and 20 μ mol g⁻¹ FW, depending on the weed species. Although there are considerable differences in their sensitivity to higher BOA concentrations, *P. oleracea*, *A. retroflexus*, and *Ch. album* represent a group of species with a consistent absorption capability. The outstanding behavior and BOA tolerance of *A. theophrasti* is due to an avoidance of BOA accumulation in seedlings. It has recently been demonstrated that benzoxazinoid detoxification after uptake from soil enriched with BOA is an alternative mechanism for explaining the differential behavior of weeds in field studies (Schulz *et al.*, 2013). Our results confirm that rye mulch has phytoactive compounds able to control specific weeds selectively and to modify the spectrum of weed species in field conditions. A rye cover crop included in a suitable crop rotation may represent a sustainable weed management practice permitting a reduction in the amount of herbicides used in agroecosystems, thus limiting the environmental risks of intensive agriculture.

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