## Research Article

# Elucidating waterhemp (*Amaranthus tuberculatus*) suppression from cereal rye cover crop biomass

Jose J. Nunes[1], Nicholas J. Arneson[2], Damon Smith[3], Matt Ruark[4], Shawn Conley[5] and Rodrigo Werle[6]

[1]Graduate Student, Department of Agronomy, University of Wisconsin, Madison, WI, USA; [2]Former Outreach Program Manager, Department of Agronomy, University of Wisconsin, Madison, WI, USA; [3]Associate Professor, Department of Plant Pathology, University of Wisconsin, Madison, WI, USA; [4]Associate Professor and Extension Soil Scientist, Department of Soil Science, University of Wisconsin, Madison, WI, USA; [5]State Extension Soybean and Small Grain Specialist, Department of Agronomy, University of Wisconsin, Madison, WI, USA and [6]Associate Professor, Department of Agronomy, University of Wisconsin, Madison, WI, USA

## Abstract

Cereal rye (*Secale cereale* L.) as a cover crop can be an effective nonchemical tool for waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] suppression in crop production. Previous studies have evaluated *A. tuberculatus* suppression by cereal rye as part of weed management programs but have not investigated the underlying mechanism of suppression by the cover crop. This study aimed to investigate the effect of cereal rye biomass on *A. tuberculatus* emergence and development, and on soil environmental parameters (temperature, moisture, and light transmittance) that are key triggers of *A. tuberculatus* germination to elucidate the mechanism of suppression by the cover crop. A dose–response study was conducted under field conditions in Brooklyn and Janesville, WI, from 2021 to 2023. Cereal rye biomass from a fall-planted field was harvested at anthesis in the spring and dried to constant weight at 60 C to provide 0.0, 0.6, 1.2, 2.4, 4.8, 7.2, 9.6, and 12.0 Mg ha$^{-1}$ of dry biomass that was evenly distributed over 1.9 m$^{-2}$ plots. Increasing cereal rye biomass reduced *A. tuberculatus* height, biomass, and density. An average ED$_{50}$ of 5.2 Mg ha$^{-1}$ of biomass was needed to reduce *A. tuberculatus* density by 50%. Low levels of biomass ($\leq$2.38 Mg ha$^{-1}$) augmented *A. tuberculatus* density due to an increase in soil moisture underneath the mulch compared with bare soil. Cereal rye biomass decreased the amount of sunlight reaching the soil, which resulted in lower mean soil temperature and temperature amplitude throughout the day (9.3 and 2.7 C temperature amplitude at 0 and 12.0 Mg ha$^{-1}$, respectively). Prevention of *A. tuberculatus* germination by this thermal effect is likely the main mechanism of *A. tuberculatus* suppression from the cereal rye cover crop. Our results support biomass from cereal rye cover crop effectively suppressing *A. tuberculatus* and contributing to the integrated management of *A. tuberculatus*.

## Introduction

Waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] is regarded as one of the most troublesome weed species in the U.S. Midwest due to its high competitiveness with cash crops and prolific seed production (Schwartz et al. 2016; Steckel 2007; Van Wychen 2022). *Amaranthus tuberculatus* management has historically relied on the frequent use of herbicides in row-crop production systems, such as corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] (Duke and Powles 2008; Green 2014). The overreliance on herbicides has selected *A. tuberculatus* populations resistant to pre- and postemergence herbicides commonly adopted for their control in such production systems (Faleco et al. 2022; Heap 2023; Peterson et al. 2018).

With the rapid and constant increase in herbicide-resistant *A. tuberculatus* populations and the lack of new herbicide modes of action entering the market in the near future (Westwood et al. 2018), nonchemical management practices are of paramount importance to diversify cropping practices and help mitigate herbicide resistance (Liebman et al. 2022; Yadav et al. 2023). Cover cropping is one of the most accessible and effective nonchemical tools to be adopted as part of integrated *A. tuberculatus* management programs in soybean production systems (Osipitan et al. 2019). Cover crops suppress weed development by competing with weeds for space, light, nutrients, and water (Schramski et al. 2021). Moreover, the biomass produced by the cover crop before its termination can serve as a mulch that protects the soil and prevents weed seeds from germinating (Mohler and Teasdale 1993; Teasdale and Mohler 2000).

Cereal rye (*Secale cereale* L.) is the most common cover crop species adopted by North American farmers due to its winter hardiness and potential for rapid and high biomass accumulation in the spring before termination (CTIC/SARE/ASTA 2023). Several studies have

evaluated the adoption of cereal rye cover crop for *A. tuberculatus* suppression in corn–soybean rotation. Bish et al. (2021) studied the effect of different fall cereal rye seeding rates (0, 34, 56, 79, 110, and 123 kg ha⁻¹) on *A. tuberculatus* suppression in subsequent soybean and found that a rate of at least 56 kg ha⁻¹ was required to have consistent *A. tuberculatus* suppression. Yadav et al. (2023) reported that cereal rye associated with reduced soybean row spacing (38 vs. 76 cm) and effective *A. tuberculatus* control in the previous crop (corn) provided the best level of *A. tuberculatus* control during the soybean season. Nunes et al. (2023b) observed that a cereal rye cover crop terminated at soybean planting (planting green) reduced early-season *A. tuberculatus* density similar to the use of a preemergence herbicide program. These studies provide evidence that cereal rye can be an effective tool for *A. tuberculatus* management. Moreover, a similar trend across studies evaluating the use of cover crops for weed suppression is that delaying cover crop termination can increase biomass accumulation and improve weed suppression (Hodgskiss et al. 2022; Nunes et al. 2023b; Osipitan et al. 2019). However, such studies focused on the applicability of this practice and did not investigate the underlying mechanism of *A. tuberculatus* suppression provided by the cereal rye cover crop.

Most of the research conducted to elucidate the mechanism of weed suppression by cover crops was conducted in the 1990s (Mohler and Teasdale 1993; Teasdale and Mohler 1993, 2000). In such studies, researchers evaluated the effect of hairy vetch (*Vicia villosa* Roth) and cereal rye biomass on the suppression of several weed species and soil parameters such as moisture, temperature, and light transmittance to elucidate the cover crop suppression mechanism. The authors reported a linear relationship between the increase in cover crop biomass and the reduction of weed density (Mohler and Teasdale 1993). The cover crop biomass lowered the soil temperature by limiting sunlight from reaching the soil, and their effect combined are key factors for weed suppression depending on the biology and response of each species to light and temperature (Teasdale and Mohler 1993). Small-seeded weeds (i.e., redroot pigweed [*Amaranthus retroflexus* L.]) are more sensitive to cover crop suppression than large-seeded species (i.e., velvetleaf [*Abutilon theophrasti* Medik.]) due to the light deprivation from the cover crop mulch (Teasdale and Mohler 2000).

Despite serving as valuable references to understand the effect of cover crops on weed suppression, these studies did not include *A. tuberculatus* response to cover crops. Moreover, to our knowledge, no other research has attempted to elucidate the mechanism of *A. tuberculatus* suppression by the cereal rye cover crop. *Amaranthus tuberculatus* germination is known to positively respond to the increase in mean soil temperature and temperature fluctuation (Leon and Knapp 2004) and to light quality, specifically red-to-far red (R:FR) ratio (Leon and Owen 2003). But no other study has evaluated the effect of soil moisture on *A. tuberculatus* emergence. Therefore, this study aimed to investigate the effect of the cereal rye biomass on *A. tuberculatus* emergence and development and on soil parameters (temperature, moisture, and light transmittance) that are key triggers of *A. tuberculatus* germination in order to elucidate the mechanism of suppression by the cover crop. We hypothesize that the increase in cereal rye biomass will have a positive response to *A. tuberculatus* suppression and will also reduce soil temperature and light transmittance as the mechanism of suppression. Nevertheless, the presence of the cereal rye biomass is likely to hold moisture underneath its mulch and promote *A. tuberculatus* emergence.

## Materials and Methods

### Study Design and Establishment

The study was conducted in a commercial production field near Brooklyn, WI (BRO; 42.87°N, 89.39°W) in 2021, 2022, and 2023 and at the University of Wisconsin–Madison Cropping Systems Weed Science research site at the Rock County Farm near Janesville, WI (ROK; 42.73°N, 89.02°W) in 2022 and 2023 following a randomized complete block design with four replications. Each experimental unit consisted of a 0.9 by 2.1 m plot established on fields historically cultivated as a corn–soybean rotation. Corn was the crop grown in the growing seasons before study initiation at each site, and no crops were grown during the seasons when the study was conducted. In ROK, the study was conducted in the same experimental area in 2022 and 2023, while in BRO, adjacent fields were used for the two experimental runs. The experimental areas were tilled before the study establishment to incorporate plant residues that could interfere with the suppression of *A. tuberculatus* provided by the cereal rye biomass treatments evaluated in the study. The soil in BRO was characterized as a loam with 40% sand, 42% silt, 18% clay, 1.7% organic matter, and a pH (H₂O) of 7.0; and in ROK, as a silt loam with 20% sand, 59% silt, 21% clay, 3.9% organic matter, and a pH (H₂O) of 6.3.

The study was designed following a dose–response treatment arrangement to evaluate the effects of increasing levels of dry cereal rye biomass (0 to 12 Mg ha⁻¹) on *A. tuberculatus* emergence and development and on soil parameters (temperature, moisture, and light transmittance). To simulate the levels of biomass, the cereal rye was harvested from a fall-planted field in the spring at the anthesis stage (Zadoks growth stage 60; Zadoks et al. 1974) by cutting the plants 5 cm from the soil surface and drying to constant weight at 60 C. The cereal rye 'Aroostook' was drilled following corn silage harvest (late September) with a 19-cm row spacing (13 rows) no-till grain drill (Yetter Farm Equipment, Colchester, IL) at a seeding rate of 67 kg ha⁻¹ and a seed depth of 2.5 cm in the previous fall of each experimental year at the Arlington Agricultural Research Station near Arlington, WI (43.30°N, 89.34°W). Once dried, biomass samples were weighed to provide 9.6 ,7.2 ,4.8 ,2.4 ,1.2 ,0.6 ,0.0, and 12.0 Mg ha⁻¹ of cereal rye biomass for an experimental unit area of 1.9 m⁻². The range of cereal rye biomass treatment doses selected for this study was based on previous research on the adoption of cereal rye cover crop for weed suppression in Wisconsin. Grint et al. (2022) and Nunes et al. (2023a) reported that cereal rye accumulated biomass levels ranging from 0.3 to 12.2 Mg ha⁻¹ at termination in corn and soybean production systems depending on management practices such as cereal rye planting and termination dates. Such findings provided support for selecting the lowest and highest cereal rye doses to be adopted in this study. The biomass samples were transported to each site and evenly distributed within the respective plot's perimeter to simulate the ground coverage provided by the cereal rye cover crop after its termination. One to three days before biomass was applied to the plots, the soil of each site was lightly tilled with a field cultivator to eliminate emerged weeds and incorporate crop residue present from the previous year so that only a minimal amount of residue remained on the soil surface. As the biomass samples were distributed over the plots, a welded wire fence (5 by 10 cm mesh) measuring the same dimensions as the plots was placed over the biomass to prevent the wind from disturbing the cereal rye mulch (Supplementary Figure S1). All plots were kept weed-free by manually removing any other weed species once a week. The study

establishment dates (when the cereal rye biomass was applied) were June 2, 2021, May 31, 2022, and May 30, 2023, in BRO, and May 30, 2022, and May 31, 2023, in ROK.

### Data Collection

Air temperature (C) and precipitation (mm) data were collected in 30-min intervals at all site-years using Spectrum WatchDog 2000 Series Mini Stations (Spectrum Technologies, Aurora, IL) placed adjacent to each experimental site. Temperature and precipitation sensors were placed at a height of 1.5 m from the ground level.

### Amaranthus tuberculatus Demographics at 42 d after Study Establishment (DAE)

Demographic data were collected at 42 DAE at all site-years. Plant height was determined by averaging the height of five random plants per plot. Plant density was calculated by averaging the number of emerged plants in two 0.1-m$^{-2}$ quadrats randomly placed within each plot. The plants counted in the two 0.1-m$^{-2}$ quadrats for density were harvested, placed in paper bags, and dried to constant weight at 60 C to determine aboveground biomass.

### Effect of Cereal Rye Biomass on Amaranthus tuberculatus Cumulative Relative Emergence

*Amaranthus tuberculatus* emergence was assessed at both sites in 2022 and 2023 (data were not collected at BRO in 2021) by counting the number of emerged seedlings in one 0.1-m$^{-2}$ permanent quadrat randomly assigned in each plot at the study establishment in a similar approach to Striegel et al. (2021). All *A. tuberculatus* seedlings were counted and removed from each quadrat weekly from 7 to 70 DAE. The weekly seedlings counts ended at 70 DAE, given that no new events of emergence were observed in any site-year after this time, which corroborates with findings from Striegel et al. (2021) and Werle et al. (2014) on *A. tuberculatus* emergence period. Care was taken to retain the cereal rye biomass in place while counting and to minimize soil disturbance during seedling removal; to standardize the assessments, only *A. tuberculatus* seedlings with at least one pair of true leaves were counted and removed. Cumulative relative emergence was calculated using Equation 1 (Picapietra and Acciaresi 2021).

$$Er_i = \frac{\sum(E_7 \ldots E_{70})}{E_n} \times 100 \qquad [1]$$

where $Er_i$ is the cumulative relative emergence (%) at time $i$, $E$ is the number of *A. tuberculatus* seedlings recorded from 7 ($E_7$) to 70 DAE ($E_{70}$), and $E_n$ is the total number of *A. tuberculatus* seedlings in the corresponding experimental unit. The total number of *A. tuberculatus* seedlings recorded in each site-year used to calculate the relative and cumulative relative emergence is available in Supplementary Table S1.

### Effect of Cereal Rye Biomass on Soil Parameters

The following data were collected in both sites in 2022 and 2023 to understand the effect of cereal rye biomass on soil temperature, moisture, and light transmittance (such data were not collected at BRO-21). Soil temperature was monitored from 0 to 70 DAE in the four replications of treatments with biomass doses of 0.0, 4.8, and 12.0 Mg ha$^{-1}$ using WatchDog 1650 Micro Stations (Spectrum Technologies). Sensors were inserted at the center of each plot to record temperature readings from 0- to 7.6-cm soil depth in 30-min intervals. Soil moisture was measured using a handheld time domain reflectometry FieldScout TDR 300 Meter (Spectrum Technologies) equipped with two 7.6-cm waveguides installed vertically to average the water content over the entire 7.6-cm soil layer. Three random readings were collected from each plot weekly from 7 to 70 DAE. Light transmittance at the soil level was measured at study establishment using a LightScout Solar/Electric Quantum Meter model 3415FXSE (Spectrum Technologies). The meter measures a light range of 0 to 2,000 μmol m$^{-2}$ s$^{-1}$, defined as units of moles striking an area over time (photosynthetically active radiation). The external sensor was attached to a 20-cm wooden stake and carefully inserted underneath the cereal rye biomass to extract two light readings per experimental unit. The light readings were collected on a sunny day with minimal cloud coverage at about the same time of the day in each site (12:00 to 12:30-hour in BRO and from 13:15 to 13:45-hour in ROK) in both years.

### Statistical Analyses

All statistical analyses were conducted in R statistical software v. 4.2.1 (R Core Team 2022). Data processing and visualization were performed with the TIDYVERSE collection of packages (Wickham et al. 2019), and nonlinear regression models were fit to describe the relationship of each response variable using the DRC package (Ritz et al. 2015). For each response variable, several models were created and compared, and the best fit was selected based on the lowest Akaike information criterion (AIC), as suggested by Keshtkar et al. (2021). All candidate models and AIC values are available in Supplementary Table S2. Candidate models were selected based on the DRC package library, which includes commonly used models for regression analysis in the weed science discipline (Arsenijevic et al. 2022; Ritz et al. 2015).

The three-parameter Weibull-1 model was fit to describe the relationship between cereal rye biomass and light transmittance (0 DAE), *A. tuberculatus* plant height (42 DAE), and *A. tuberculatus* biomass (42 DAE) for each site-year. The same model was also adopted to explain *A. tuberculatus* cumulative relative emergence under different cereal rye biomass doses as a function of the day of the year. For *A. tuberculatus* cumulative relative emergence, the data from both sites and years were pooled together due to the similarities in response across site-years. The three-parameter Weibull-1 model has its lower limit fixed at zero and is represented by Equation 2 (Ritz et al. 2015):

$$y = 0 + (d - 0)\exp(-\exp\{b[\log(x) - \log(e)]\}) \qquad [2]$$

where $y$ is the response variable, $x$ is the cereal rye biomass dose or day of the year for *A. tuberculatus* cumulative relative emergence, $b$ is the relative slope at the inflection point, $d$ is the upper limit or asymptote, and $e$ is the inflection point of the curve.

The four-parameter Cedergreen-Ritz-Streibig model was fit to describe *A. tuberculatus* density as a function of cereal rye biomass for each site-year at 42 DAE. Similar to the three-parameter Weibull model, the four-parameter Cedergreen-Ritz-Streibig also has its lower limit fixed at zero and is represented by Equation 3 (Ritz et al. 2015):

$$y = 0 + \frac{d - 0 + f\exp(-1/x)}{1 + \exp\{b[\log(x) - \log(e)]\}} \qquad [3]$$

where $y$ is the response variable, $x$ is the cereal rye biomass dose, $b$ and $e$ do not have a direct interpretation, $d$ is the upper limit or

asymptote, and *f* denotes the size of the hormesis effect. The larger the value of *f*, the larger the hormesis effect; *f* = 0 corresponds to no hormesis effect. The Cedergreen-Ritz-Streibig model is often used to describe hormetic responses (Ritz et al. 2015), which are characterized by a low-dose response that is opposite in effect to that seen at high doses (Mattson 2007), a condition observed in this study when higher *A. tuberculatus* densities were observed in low levels of cereal rye biomass compared with the absence of biomass. The maximum cereal rye biomass dose, which resulted in an increase in *A. tuberculatus* density before it began to decrease, was estimated using the function *MAX()* (DRC package; Ritz et al. 2015).

Relative response index (RRI) was calculated to standardize and compare *A. tuberculatus* response variables (density, plant height, and biomass at 42 DAE) as a function of cereal rye biomass. The RRI expresses plant response to cover crop residues in relation to the bare soil control, as determined using Equation 4 (Williams et al. 1998):

$$RRI = (P_{cn} - P_r)/(P_{cn} + P_r) \qquad [4]$$

where $P_{cn}$ represents plant response (*A. tuberculatus* density, plant height, and biomass) in the bare soil control, which was given by the average of the four observations of each variable in the biomass dose zero from each site-year; and $P_r$ is plant response (*A. tuberculatus* density, plant height, and biomass) in a cereal rye biomass treatment. An RRI value greater than 0 indicates that the biomass decreased plant fitness; if RRI is equal to 0, the biomass had no effect on plant fitness; and if RRI is less than 0, the biomass increased plant fitness. The four-parameter log-logistic model (Equation 4) was fit to the calculated RRI values with the parameter *d* (upper limit) fixed at 1. The data of all 5 site-years were combined to create one single model focusing on the effect of cereal biomass on the response variables without the effect of site-year. The cereal rye biomass dose 0 was not included in the dose–response model, as it would have effected the estimation of parameter *c* (lower limit).

The four-parameter log-logistic model was fit to RRI and soil volumetric water content data (average of 10 readings from 7 to 70 DAE) of each site-year. The model is represented by Equation 5 (Ritz et al. 2015):

$$y = c + \frac{d - c}{1 + \exp\{b[\log(x) - \log(e)]\}} \qquad [5]$$

where *y* is the response variable; *x* is the cereal rye biomass dose; *b* is the relative slope at the inflection point; *c* and *d* are the lower and upper limits or asymptotes, respectively; and *e* is the inflection point of the curve.

The effective dose of cereal rye biomass required to achieve 50% (absolute $ED_{50}$) cumulative relative emergence and RRI, or 50% reduction in *A. tuberculatus* biomass, plant height, density, and light transmittance, was calculated using the *ED()* function (DRC package; Ritz et al. 2015). Moreover, model parameters, such as upper or lower limits, were compared using the *compParm()* function (DRC package; Ritz et al. 2015) to assist the discussion of results when necessary. The *compParm()* function runs pairwise *t*-tests to compare model parameters and test whether the parameters are significantly different or not based on the model and data (Ritz et al. 2015).

Soil temperature was the variable that required the most data processing before analysis. A daily average for each hour (h) of the day and dose of cereal rye biomass (0.0, 4.8, and 12.0 Mg ha$^{-1}$) was calculated by averaging the 70 d of temperature readings collected in each site-year. Because the soil temperature was only measured at cereal rye doses of 0.0, 4.8, and 12.0 Mg ha$^{-1}$, a polynomial regression with a quadratic term was used to estimate the soil temperature for the remaining biomass doses based on the data recorded at those three doses. Moreover, due to the similar response in temperature across site-years (visual assessment of soil temperature across site-years), the data of both sites and years (4 site-years) were combined to generate a data set with four observations of hourly average temperature for each cereal rye biomass dose. This data set was then used to fit polynomial regression models with a quadratic term for each hour of the day to explain soil temperature as a function of cereal rye biomass. All models were fit using the *lm* function in R software, and the assumptions of normality and homogeneity of variance were assessed by visual inspection of residuals.

## Results and Discussion

Precipitation was the environmental factor that varied the most across site-years during the time span of the study (Figure 1). The lowest precipitation was recorded in 2023, when a total of 126 mm was recorded in ROK-23 (site ROK year 2023) compared with 184 mm in ROK-22. The lower precipitation in BRO was offset by an irrigation system that supplemented precipitation in 2023. A total of 81 mm applied through irrigation in BRO-23 combined with 117 mm of rainfall yielded a total of 198 mm in 2023. In 2021 and 2022, totals of 219 and 215 mm were recorded in BRO, respectively (no irrigation applied in these 2 yr). As a result of the low precipitation, *A. tuberculatus* density was also the lowest in 2023 at both sites. Although the irrigation system contributed to the total precipitation recorded in BRO-23, *A. tuberculatus* emergence was still lower than usual at this research site (JJN, personal observation). Air temperature patterns were fairly similar across all site-years, with mean temperature averaging from 18.9 (BRO-22) to 21.4 C (BRO-21) (Supplementary Figure S2). The minimum, mean, and maximum temperatures, respectively, recorded at each site-year were BRO-21 (5.6, 21.4, and 26.7 C), BRO-22 (5.7, 18.9, and 25.9 C), BRO-23 (11.4, 20.6, and 25.6 C), ROK-22 (11.7, 21.3, and 29.4 C), and ROK-23 (11.5, 21.0, and 26.1 C).

It is important to emphasize that this study focused on the physical influence of the cereal rye biomass on *A. tuberculatus* suppression and soil microenvironment (light, moisture, and temperature). The methodology adopted simulates the mulch effect of the cereal rye biomass following its termination and during decomposition, which is commonly observed when cereal rye is adopted ahead of soybean (Nunes et al. 2023b). Because there was no cereal rye growth in the experimental area where the study was conducted, this study did not account for the effect of the cover crop on water (Reed and Karsten 2022) and nutrient (Finney et al. 2016) dynamics during its growth and their potential impact on *A. tuberculatus* recruitment (Boyd and Van Acker 2003; Sweeney et al. 2008). Additionally, because the goal of the study was to focus on the physical effect of the cereal rye mulch, we did not measure the activity of allelochemicals on *A. tuberculatus* suppression (Burgos and Talbert 2000). Further information on those aspects of weed suppression by cereal rye can be found in a literature review by Camargo Silva and Bagavathiannan (2023).
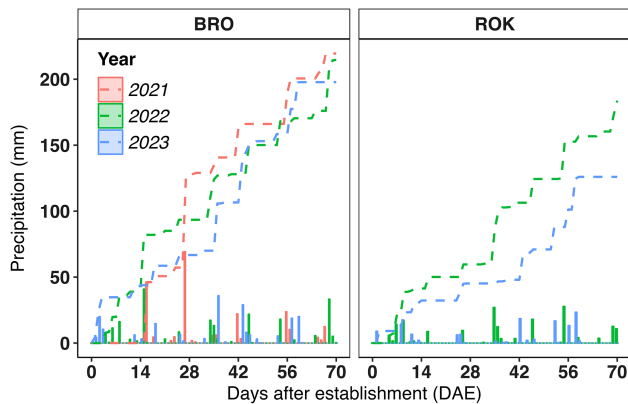
**Figure 1.** Daily (bars) and cumulative (dashed lines) precipitation (mm) from 0 to 70 d after establishment (DAE) in Brooklyn, WI (BRO) in 2021 (219 mm total), 2022 (215 mm total), and 2023 (198 mm total), and in Janesville, WI (ROK) in 2022 (184 mm total) and 2023 (126 mm total). Note that the study was concluded within 42 DAE in BRO in 2021. Irrigation was used in BRO in 2023 only, and was applied at 30, 15, and 36 mm at 3, 18, and 36 DAE, respectively.

## *Amaranthus tuberculatus* Suppression at 42 DAE

Cereal rye biomass provided effective *A. tuberculatus* suppression by reducing its height, biomass, and density at 42 DAE (Figures 2–4). For all three response variables, there was an inverse relationship with the increase in cereal rye biomass. *Amaranthus tuberculatus* biomass had a similar response to the increase in cereal rye doses across site-years and required the lowest overall doses of biomass (1.76 to 3.35 Mg ha$^{-1}$) to achieve a 50% reduction in plant weight compared with plant height and density (Tables 1 and 2). Conversely, *A. tuberculatus* height varied across site-years, specifically in BRO-23, where plant height was overall higher across site-years, which is likely due to the lower *A. tuberculatus* density observed in BRO-23 compared with all other site-years. Lower *A. tuberculatus* density likely yielded lower intraspecific competition between *A. tuberculatus* plants, hence higher growth of individual plants. As a result, the ED$_{50}$ to achieve a 50% reduction in *A. tuberculatus* height was highest (6.95 Mg ha$^{-1}$) in BRO-23 and varied between 1.04 and 5.48 Mg ha$^{-1}$ for the remaining site-years.

*Amaranthus tuberculatus* density showed an intriguing response to the increase in cereal rye biomass through augmented emergence under low doses ($\leq 2.38$ Mg ha$^{-1}$) compared with the absence of biomass in all site-years (Figure 4). A similar trend was observed by Teasdale and Mohler (2000), who reported an increase in *A. retroflexus* emergence under low levels (<2.0 Mg ha$^{-1}$) of a legume mulch composed of a mixture of hairy vetch and crimson clover (*Trifolium incarnatum* L.). The Cedergreen-Ritz-Streibig model, often used to describe hormesis (Ritz et al. 2015), was adopted to explain this effect and the relationship between *A. tuberculatus* density and cereal rye biomass. Across the 5 site-years of data, the hormesis effect was significant ($f \neq 0$; P < 0.05) in 3 site-years (BRO-22, ROK-22, and ROK-23; Table 3). Although the hormesis effect was nonsignificant ($f = 0$; P > 0.05) in the other 2 site-years, the Cedergreen-Ritz-Streibig model still provided a better fit than other models such as Weibull and log-logistic. One hypothesis behind the increase in *A. tuberculatus* emergence under low levels of cereal rye is that the biomass could increase soil moisture underneath its mulch, which stimulates weed emergence compared with bare ground (Mohler and Teasdale 1993; Teasdale and Mohler 2000; Williams et al. 1998). To test this hypothesis, soil moisture was measured weekly from 7 to 70 DAE, and the average
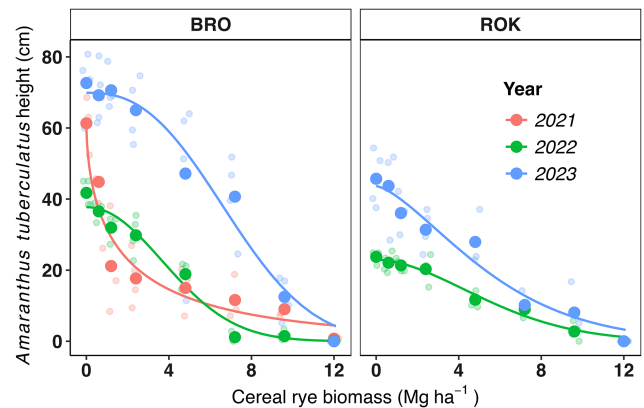


**Figure 2.** *Amaranthus tuberculatus* plant height (cm) as a function of cereal rye biomass (Mg ha$^{-1}$) at 42 d after establishment (DAE) in *Brooklyn, WI (BRO) in 2021, 2022, and 2023, and Janesville, WI (ROK) in 2022 and 2023*. Lines represent the model fit; large dark symbols represent *A. tuberculatus* plant height means within each cereal rye biomass level; and small light-colored symbols represent individual observations.
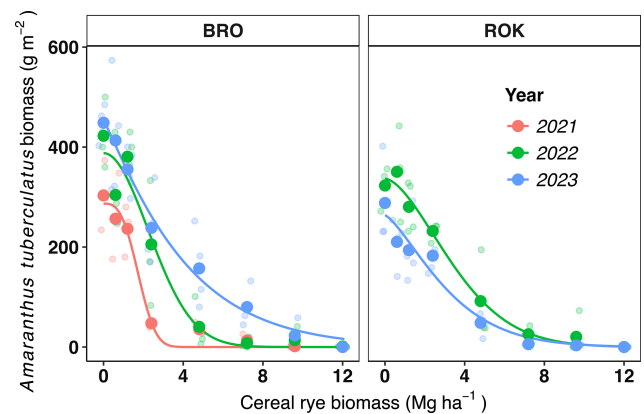


**Figure 3.** *Amaranthus tuberculatus* biomass (g m$^{-2}$) as a function of cereal rye biomass (Mg ha$^{-1}$) at 42 d after establishment (DAE) in *Brooklyn, WI (BRO) in 2021, 2022, and 2023, and Janesville, WI (ROK) in 2022 and 2023*. Lines represent the model fit; large dark symbols represent *A. tuberculatus* aboveground biomass means within each cereal rye biomass level; and small light-colored symbols represent individual observations.
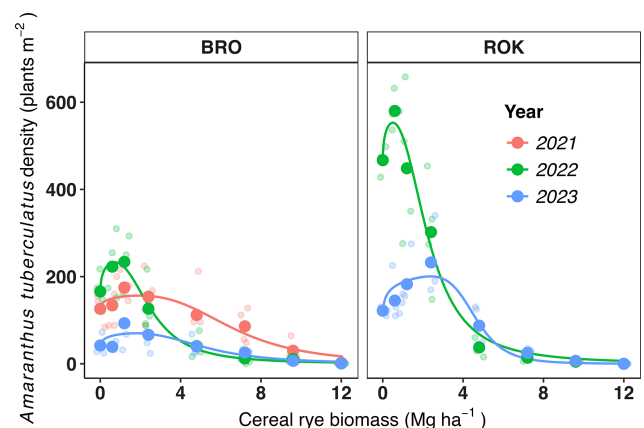


**Figure 4.** *Amaranthus tuberculatus* density (plants m$^{-2}$) as a function of cereal rye biomass (Mg ha$^{-1}$) at 42 d after establishment (DAE) in *Brooklyn, WI (BRO) in 2021, 2022, and 2023, and Janesville, WI (ROK) in 2022 and 2023*. Lines represent the model fit; large dark symbols represent *A. tuberculatus* density means within each cereal rye biomass level; and small light-colored symbols represent individual observations.

**Table 1.** Weibull-1 model parameter estimates and standard errors (SE) for slope (*b*), upper limit (*d*), and inflection point (*e*) for *Amaranthus tuberculatus* plant height (cm), *Amaranthus tuberculatus* biomass (g m$^{-2}$), and light transmittance ($\mu$mol m$^{-2}$ s$-1$) in Brooklyn, WI (BRO) in 2021, 2022, and 2023, and Janesville, WI (ROK) in 2022 and 2023

| | BRO | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2021 | | | 2022 | | | 2023 | | |
| Response variable | *b* (±SE) | *d* (±SE) | *e* (±SE) | *b* (±SE) | *d* (±SE) | *e* (±SE) | *b* (±SE) | *d* (±SE) | *e* (±SE) |
| Plant height | 0.55[a] (0.08) | 62.0 (3.7) | 2.03 (0.38) | 2.01 (1.01) | 37.7 (3.3) | 5.10 (0.62) | 2.54 (0.42) | 69.9 (2.2) | 8.03 (0.33) |
| Biomass | 2.98 (1.12) | 286.9 (21.9) | 1.98 (0.19) | 1.99 (0.53) | 387.8 (21.2) | 3.15 (0.28) | 1.12 (0.15) | 452.7 (22.7) | 4.11 (0.41) |
| Light transmittance | | | | 1.12 (0.11) | 1,721.9 (41.9) | 0.92 (0.04) | 0.88 (0.07) | 1,688.7 (41.8) | 1.46 (0.08) |

| | ROK | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2021[b] | | | 2022 | | | 2023 | | |
| | *b* (±SE) | *d* (±SE) | *e* (±SE) | *b* (±SE) | *d* (±SE) | *e* (±SE) | *b* (±SE) | *d* (±SE) | *e* (±SE) |
| Plant height | — | — | — | 1.81 (0.82) | 22.9 (2.6) | 6.60 (1.17) | 1.46 (0.43) | 43.6 (3.3) | 6.27 (0.76) |
| Biomass | — | — | — | 1.67 (0.38) | 336.7 (19.80) | 4.17 (0.43) | 1.37 (0.54) | 263.4 (28.9) | 3.56 (0.64) |
| Light transmittance | — | — | — | 1.85 (0.19) | 1,931.3 (42.0) | 0.90 (0.02) | 0.90 (0.05) | 1,947.9 (41.9) | 1.66 (0.08) |

[a]All model parameters were statistically different from zero (P < 0.05).
[b]Study not conducted at this site in 2021.

**Table 2.** Estimated effective dose (ED$_{50}$) and standard errors (SE) of cereal rye biomass (Mg ha$^{-1}$) to achieve 50% reduction in *Amaranthus tuberculatus* biomass (g m$^{-2}$), *Amaranthus tuberculatus* plant height (cm), *Amaranthus tuberculatus* density (plants m$^{-2}$), and light transmittance ($\mu$mol m$^{-2}$ s$^{-1}$) in Brooklyn, WI (BRO) in 2021, 2022, and 2023, and Janesville, WI (ROK) in 2022 and 2023

| | BRO | | | ROK | |
|---|---|---|---|---|---|
| Response variable | 2021 | 2022 | 2023 | 2022 | 2023 |
| | — Mg ha$^{-1}$ — | | | | |
| Plant height | 1.04 (0.26) | 4.25 (0.80) | 6.95 (0.38) | 5.38 (1.23) | 4.89 (0.85) |
| Biomass | 1.76 (0.21) | 2.63 (0.26) | 2.97 (0.37) | 3.35 (0.41) | 2.72 (0.70) |
| Density | 7.54 (1.08) | 3.22 (0.51) | 7.23 (3.08) | 2.60 (0.17) | 5.40 (0.66) |
| Light transmittance | —[a] | 0.67 (0.04) | 0.97 (0.07) | 0.74 (0.03) | 1.10 (0.07) |

[a]Data not collected at this site in 2021.

**Table 3.** Cedergreen-Ritz-Streibig model parameter estimates and standard errors (SE) for *b* and *e*, upper limit (*d*), hormesis effect (*f*) for *Amaranthus tuberculatus* density (plants m$^{-2}$), and the maximum dose of cereal rye biomass (Mg ha$^{-1}$) to result in hormesis effect in Brooklyn, WI (BRO) in 2021, 2022, and 2023, and Janesville, WI (ROK) in 2022 and 2023[a]

| | BRO | | | | | |
|---|---|---|---|---|---|---|
| Year | *b* (±SE) | *d* (±SE) | *e* (±SE) | *f* (±SE) | P[b] | Maximum |
| | | | | | | —Mg ha$^{-1}$— |
| 2021 | 3.61 (1.63) | 124.7 (24.4) | 6.53 (1.19) | 78.4 (79.2) | 0.324 | 1.94 |
| 2022 | 2.88 (0.96) | 164.7 (24.6) | 2.42 (0.39) | 223.5 (106.7) | 0.038 | 0.72 |
| 2023 | 3.29 (2.66) | 38.3 (24.3) | 5.04 (1.99) | 80.9 (80.2) | 0.314 | 1.77 |

| | ROK | | | | | |
|---|---|---|---|---|---|---|
| | *b* (±SE) | *d* (±SE) | *e* (±SE) | *f* (±SE) | P | Maximum |
| | | | | | | —Mg ha$^{-1}$— |
| 2022 | 2.69 (0.48) | 470.4 (24.7) | 2.20 (0.26) | 304.7 (148.3) | 0.042 | 0.50 |
| 2023 | 6.47 (3.86) | 111.5 (24.1) | 4.59 (0.35) | 205.3 (72.5) | 0.005 | 2.38 |

[a]Except for the parameters *b* for BRO (P = 0.210) and ROK (P = 0.096) in 2023 and *d* for BRO in 2023 (P = 0.118), all other *b*, *d*, and *e* parameters were statistically different from zero (P < 0.05).
[b]P to test whether the parameter *f* (hormesis effect) is different from zero ($\alpha$ = 0.05).

of the 10 readings showed a positive relationship with the increase in cereal rye biomass (Table 4; Figure 5). At the lowest cereal rye dose of 0.6 Mg ha$^{-1}$, an average of 5.2% increase in soil moisture was observed compared with the absence of biomass. Moreover, Figure 5 shows that ROK-23 was the site year with the lowest overall soil moisture compared with all other site-years, which can be explained by the low precipitation in ROK-23 (Figure 1). ROK-23 was also the site-year with the highest maximum dose of cereal rye biomass (2.38 Mg ha$^{-1}$) to provide an increase in emergence of *A. tuberculatus* across site-years (Table 3). Thus, these results support the hypothesis that the increase in soil moisture underneath the cereal rye mulch can stimulate and increase *A. tuberculatus* density compared with bare soil up to a limit ($\leq$2.38 Mg ha$^{-1}$) at which the biomass level becomes high enough to provide suppression. It should be noted that soil moisture was similar in all treatments at the initiation of the study and that cereal

**Table 4.** Log-logistic model parameter estimates and standard errors (SE) for slope (*b*), lower limit (*c*), upper limit (*d*), and inflection point (*e*) for *soil volumetric water content (m³ m⁻³)* in Brooklyn, WI (BRO) and Janesville, WI (ROK) in 2022 and 2023

| Site | Year | Model parameters[a] | | | |
|------|------|---------|---------|---------|---------|
| | | *b* (±SE) | *c* (±SE) | *d* (±SE) | *e* (±SE) |
| BRO | 2022 | −3.88 (2.10) | 19.9 (0.5) | 27.9 (0.8) | 3.99 (0.55) |
| BRO | 2023 | −1.89 (0.85) | 19.5 (0.7) | 33.3 (4.0) | 5.60 (1.99) |
| ROK | 2022 | −1.20 (0.37) | 20.4 (0.8) | 46.9 (10.7) | 7.79 (5.46) |
| ROK | 2023 | −3.58 (1.18) | 13.8 (0.5) | 27.2 (1.1) | 4.55 (0.39) |

[a]Except for the parameters *b* for BRO (P 0.064) and *e* for ROK (P 0.157) in 2022, all other *b*, *c*, *d*, and *e* parameters were statistically different from zero (P < 0.05).
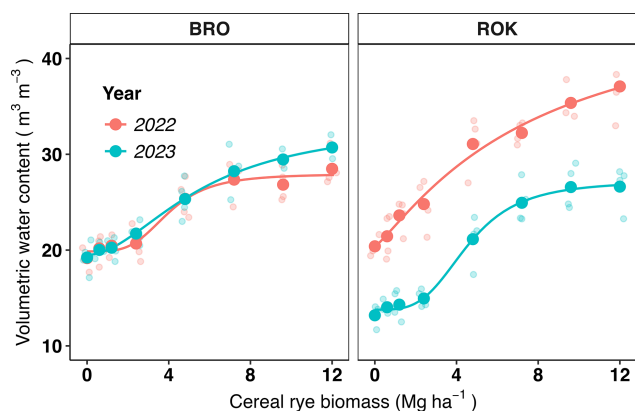


**Figure 5.** Soil volumetric water content (m³ m⁻³; 7.6-cm depth) as a function of cereal rye biomass (Mg ha⁻¹) in Brooklyn, WI (BRO) and Janesville, WI (ROK) in 2022 and 2023. An average of 10 readings performed weekly from 7 to 70 d after establishment. Lines represent the model fit; large dark symbols represent volumetric water content means within each cereal rye biomass level; and small light-colored symbols represent individual observations.

rye biomass can reduce water evaporation from the soil after its termination and increase soil moisture underneath its mulch, which is the condition being studied herein. Nevertheless, before termination, cereal rye can reduce soil moisture under dry weather spells due to its water use (Reed and Karsten 2022).

Employing a range of cover crop biomass levels wide enough to fit regression models and calculate $ED_{50}$ for weed density, biomass, and height in the same study is not a commonly used methodology to investigate weed suppression by cover crops. Thus, $ED_{50}$ values from other studies are not readily available for comparison. Conversely, meta-analysis has become a more common approach to draw inferences on the effect of increasing rates of cover crop biomass on weed suppression. Nichols et al. (2020) is an example of such approach; those authors summarized studies conducted in the U.S. Midwest and reported that 5 Mg ha⁻¹ of cover crop biomass is needed to achieve a 50% reduction in weed biomass. However, their analysis revealed that cover crops did not significantly reduce weed density. Weisberger et al. (2023) summarized studies from the U.S. Southeast and found that cover crops only reduced weed density but not biomass. Based on their findings, 6.6 Mg ha⁻¹ of cover crop biomass is required to achieve a 50% reduction in weed density. Osipitan et al. (2019) summarized studies from North America, Europe, Asia, and South America and found a linear relationship between the increase in cover crop biomass and the reduction of weed density and biomass. Such disparities in the findings of each meta-analysis emphasize the applicability of the methodology used herein to make inferences on weed suppression

by cover crops. By evaluating *A. tuberculatus* suppression under levels of cereal rye biomass wide enough to fit regression models, we were able to isolate the effect of biomass without the variability that is typical with meta-analysis due to the differences in environmental factors among the studies being compared. Thus, future studies working with different cover crop and weed species can be conducted and results compared through the use of more appropriate regression models. Moreover, the estimated ED values can serve as a target for farmers adopting cereal rye for weed suppression to adapt their management practices to accumulate the desired level of biomass for effective weed suppression.

## RRI

The RRI allowed us to compare the three response variables (*A. tuberculatus* height, density, and biomass) collected at 42 DAE and infer which variable was most impacted by the cereal rye biomass (Table 5; Figure 6). For *A. tuberculatus* biomass and height, all cereal rye doses decreased plant fitness and negatively affected these two response variables, which is evidenced by the positive lower limit (*c*) of both curves, estimated at 0.09 and 0.14, respectively, and different from zero (P < 0.05). A positive lower limit indicates that even the lowest cereal rye dose (0.6 Mg ha⁻¹) already decreased *A. tuberculatus* fitness as evidenced by these two variables. As the biomass doses increased, higher RRI values were observed, reflecting the reduction in *A. tuberculatus* biomass and height by the cereal rye. *Amaranthus tuberculatus* biomass was the most affected variable and required the lowest $ED_{50}$ (2.97 Mg ha⁻¹) to achieve a 50% increment in RRI compared with height (5.44 Mg ha⁻¹) and density (6.27 Mg ha⁻¹). Conversely, *A. tuberculatus* density presented a negative lower limit (*c* = −0.10) different from zero (P < 0.05), which implies that the cereal rye biomass increased plant fitness at the lowest dose of 0.6 Mg ha⁻¹. This response was expected, as low cereal rye doses (≤2.38 Mg ha⁻¹) increased *A. tuberculatus* density (Figure 4). Figure 6 shows that up to 2.4 Mg ha⁻¹, the RRI was either negative or close to zero but became positive with cereal rye doses greater than 4.8 Mg ha⁻¹. Using RRI allowed us to infer that cereal rye alone might not be beneficial for weed suppression depending on the biomass level. Thus, management practices, such as cereal rye planting and termination dates, should be taken into consideration when adopting this practice to optimize biomass accumulation and reach levels that will likely provide effective *A. tuberculatus* suppression. The RRI can also be used to calculate the response comparison index that quantifies the difference between the RRI of two species to determine which species is favored under cover crop treatments (Williams et al. 1998). Because only *A. tuberculatus* was studied, such comparisons are not feasible, but values reported herein may serve as a reference for future studies.

**Table 5.** Log-logistic model parameter estimates and standard errors (SE) for slope (*b*), lower limit (*c*), inflection point (*e*), and effective dose (ED$_{50}$) for the relative response index (RRI) of *Amaranthus tuberculatus* density (plants m$^{-2}$), *Amaranthus tuberculatus* height (cm), and *Amaranthus tuberculatus* biomass (g m$^{-2}$)

| Response variable | Model parameters[a] | | | |
| | *b* (±SE) | *c* (±SE) | *e* (±SE) | ED$_{50}$ (±SE) |
| --- | --- | --- | --- | --- |
| Density | −3.04 (0.33) | −0.10 (0.03) | 5.57 (0.27) | 6.27 (0.27) |
| Plant height | −3.50 (0.55) | 0.14 (0.03) | 6.61 (0.35) | 5.44 (0.41) |
| Biomass | −2.51 (0.33) | 0.09 (0.04) | 3.43 (0.29) | 2.97 (0.29) |

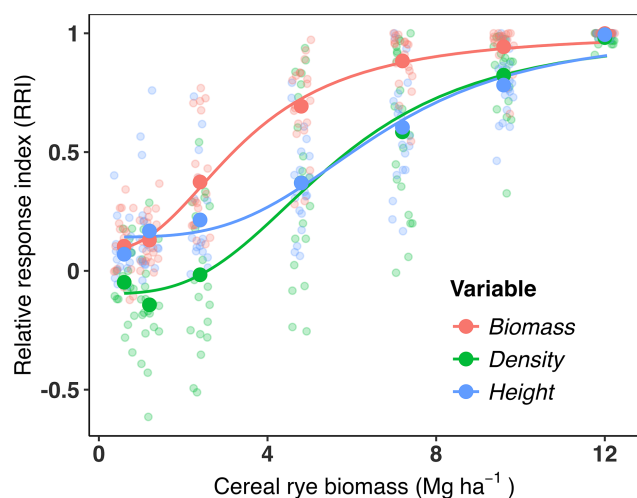[a]All model parameters were statistically different from zero (P < 0.05).



**Figure 6.** Relative response index (RRI) for *Amaranthus tuberculatus* biomass (g m$^{-2}$), density (plants m$^{-2}$), and plant height (cm) as a function of cereal rye biomass (Mg ha$^{-1}$). Data pooled across all site-years. Lines represent the model fit; large dark symbols represent RRI means within each cereal rye biomass level; and small light-colored symbols represent individual observations.
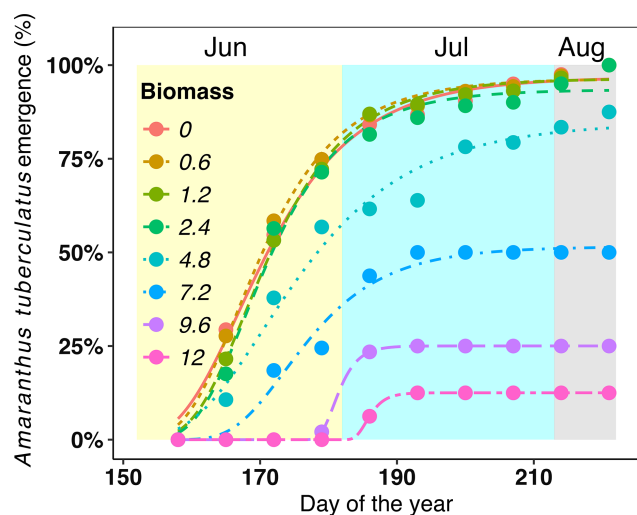


**Figure 7.** Cumulative *Amaranthus tuberculatus* emergence (%) over time for each dose of cereal rye biomass (Mg ha$^{-1}$) as a function of the day of the year. Data pooled across all site-years. Lines represent the model fit; and symbols represent cumulative *A. tuberculatus* emergence means.

### *Amaranthus tuberculatus* Cumulative Relative Emergence

*Amaranthus tuberculatus* cumulative relative emergence was evaluated to infer whether the cereal rye biomass could not only reduce but also delay *A. tuberculatus* emergence. Figure 7 illustrates *A. tuberculatus* cumulative relative emergence under

the different doses of cereal rye biomass. For biomass doses ≤4.8 Mg ha$^{-1}$, the first *A. tuberculatus* emergence event was recorded at 14 DAE and rapidly increased until the beginning of July, when it reached a slower increase rate until 70 DAE. The dose of 4.8 Mg ha$^{-1}$ provided a slight reduction in the percent of emerged seedlings at all weekly assessments, but its upper limit (*d* = 84.9) was still similar (P > 0.05) to the upper limit (*d* ≤ 96.9) of biomass doses ≤2.4 Mg ha$^{-1}$ (Table 6). The upper limit in this context indicates the estimated total percentage of emergence at the end of the study, which was estimated at ≥84.9% for biomass doses ≤4.8 Mg ha$^{-1}$. Conversely, cereal rye biomass levels ≥7.2 Mg ha$^{-1}$ delayed the onset of *A. tuberculatus* emergence for at least 7 d (first event at 21 DAE) and resulted in an upper limit (*d* ≤ 51.6) that was significantly lower (P < 0.05) than all cereal rye doses below this threshold. An upper limit of 51.6 indicates that for biomass doses ≥7.2 Mg ha$^{-1}$, *A. tuberculatus* did not emerge in about 50% of the experimental units across all site-years. Thus, total cumulative relative emergence did not reach the levels recorded at biomass levels ≤4.8 Mg ha$^{-1}$ where *A. tuberculatus* emergence happened in all experimental units. Moreover, the ED$_{50}$ (estimated day of the year to achieve 50% cumulative relative emergence based on each curve) reveals that biomass doses of ≥7.2 Mg ha$^{-1}$ delayed by at least 6 d the achievement of 50% emergence compared with the absence of cereal rye. A 50% *A. tuberculatus* emergence would be estimated to happen after June 26 of each year that the study was conducted (Table 6). A similar trend was observed by Mohler and Teasdale (1993), who reported that the mean emergence date of several weed species was delayed by more than a week by cereal rye and hairy vetch biomass.

Our findings bring new insights into the benefits of the cereal rye cover crop for *A. tuberculatus* suppression. Besides the density reduction provided by the cereal rye, which has already been reported in other studies (Bish et al. 2021; Cornelius and Bradley 2017; Nunes et al. 2023b), delaying the beginning of *A. tuberculatus* emergence by levels of biomass ≥7.2 Mg ha$^{-1}$ can also serve as an additional benefit for farmers adopting this practice. The delay in *A. tuberculatus* emergence, combined with lower *A. tuberculatus* density, can allow extra time for farmers to deploy management practices after cash crop planting, such as application of postemergence herbicides. Also, the delay in *A. tuberculatus* emergence may also reduce the number of postemergence herbicide applications required in row-crop production systems, such as corn and soybean, when timed with the canopy closure.

### Effect of Cereal Rye Biomass on Soil Parameters

The effect of cereal rye biomass on soil parameters was evaluated to extract information on environmental factors that could trigger or affect *A. tuberculatus* emergence and help elucidate the mechanism of suppression by the cereal rye biomass. As previously discussed, soil moisture had a positive effect on *A. tuberculatus* density by

**Table 6.** Weibull-1 model parameter estimates and standard errors (SE) for slope (*b*), upper limit (*d*), and inflection point (*e*) for *Amaranthus tuberculatus* cumulative relative emergence (%) under different cereal rye biomass doses

| Cereal rye biomass | Model parameters[a] | | | ED$_{50}$ (±SE)[b] | Date |
|---|---|---|---|---|---|
| | *b* (±SE) | *d* (±SE) | *e* (±SE) | | |
| —Mg ha$^{-1}$— | | | | Days[c] | |
| 0.0 | −18.4 (3.8) | 96.9 (4.3) | 167.3 (1.3) | 171 (1.6) | June 20 |
| 0.6 | −20.9 (4.4) | 96.4 (3.8) | 166.9 (1.2) | 170 (1.4) | June 19 |
| 1.2 | −21.1 (4.5) | 96.4 (3.9) | 168.2 (1.2) | 171 (1.4) | June 20 |
| 2.4 | −22.1 (5.4) | 93.5 (4.0) | 168.1 (1.2) | 171 (1.4) | June 20 |
| 4.8 | −15.3 (4.1) | 84.9 (6.1) | 171.1 (1.8) | 175 (2.5) | June 24 |
| 7.2 | −22.3 (7.8) | 51.6 (4.0) | 173.9 (2.3) | 177 (2.5) | June 26 |
| 9.6 | −93.9 (128.5) | 25.0 (3.1) | 180.8 (3.1) | 181 (3.7) | June 30 |
| 12.0 | −122.6 (1,558.7) | 12.5 (3.3) | 185.4 (7.3) | 186 (2.6) | July 5 |

[a]Except for the parameters *b* for the biomass levels of 9.6 (P 0.465) and 12.0 (P 0.937), all other *b*, *d*, and *e* parameters were statistically different from zero (P < 0.05).
[b]Effective dose (ED$_{50}$) estimates the day of the year to achieve 50% *Amaranthus tuberculatus* emergence and the date that respective day of the year represents.
[c]Models were based on the 157th day of the year (June 7 of 2022 and 2023; the date of the first assessment in each site) as the initial point.

stimulating its emergence at low (≤2.38 Mg ha$^{-1}$) cereal rye doses. The two remaining variables, light transmittance and soil temperature, were highly impacted by the increase in cereal rye biomass (Figures 8 and 9). For light transmittance, between 0.67 to 1.10 Mg ha$^{-1}$ of biomass was needed to achieve a 50% reduction in light at the soil level compared with bare ground across site-years (Table 2). Teasdale and Mohler (1993) observed that 1.52 Mg ha$^{-1}$ of cereal rye biomass was needed for a 50% reduction in light transmittance, indicating that even at extremely low levels, cereal rye biomass can intercept a great portion of the sunlight reaching the soil. Nevertheless, Teasdale and Mohler (1993) reported that despite reducing light quantity, the cereal rye biomass had very little influence on the quality (R:FR ratio) of the light transmitted through the residue.

As for soil temperature, the presence of cereal rye biomass reduced the mean temperature and the temperature amplitude, mainly by reducing the maximum temperature recorded during the day (Figure 9; Supplementary Table S3). This effect has been previously described by Teasdale and Mohler (1993), who reported that hairy vetch biomass affected the maximum soil temperature to a larger extent than the minimum temperature. For all three levels of cereal rye biomass, 0.0, 4.8, and 12.0 Mg ha$^{-1}$, the maximum soil temperature was observed at 1600 hours, and reached averages of 28.3, 24.0, and 22.4 C, respectively. Conversely, the minimum soil temperature was recorded between 0600 and 0700 hours, and reached averages of 19.0, 19.5, and 19.7 C for biomass doses of 0.0, 4.8, and 12.0 Mg ha$^{-1}$, respectively.

The impact of the cereal rye biomass on light transmittance and soil temperature shows that cereal rye biomass levels greater than 4.8 Mg ha$^{-1}$ limit most of the sunlight reaching the soil and keep it cooler for most of the day and warmer for a shorter period compared with bare soil (Figure 10). Consequently, the temperature fluctuation throughout the day becomes lower as the level of biomass protecting the soil from sunlight increases. The effect of alternating temperature has been previously described as an important factor for *A. tuberculatus* germination (Leon and Knapp 2004; Steckel et al. 2004). Leon and Knapp (2004) reported that *A. tuberculatus* germination increased with the increase in mean temperature and amplitude of temperature alternation. The percent *A. tuberculatus* germination went from 32% at 0 C amplitude of diurnal temperature alternation to 48%, 73%, 90%, and 95% at 6, 12, 18, and 24 C amplitudes, respectively (Leon and Knapp 2004). Steckel et al. (2004) observed a similar trend in the effect of alternating temperature on the increase of *A. tuberculatus*
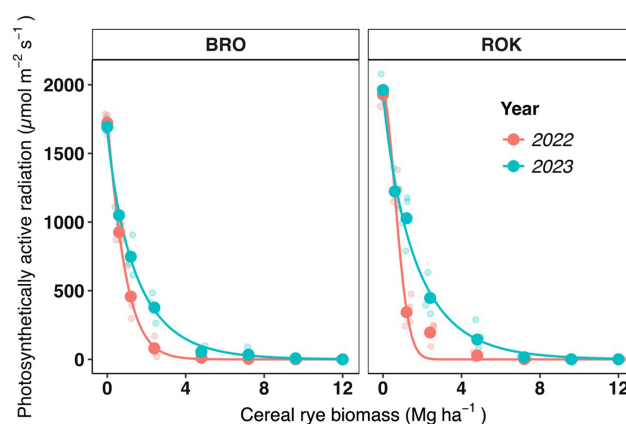


**Figure 8.** Light transmittance (*μmol m$^{-2}$ s$^{-1}$*) at the soil level as a function of cereal rye biomass (Mg ha$^{-1}$) at study establishment in *Brooklyn, WI (BRO) and Janesville, WI (ROK) in 2022 and 2023*. Lines represent the model fit; large dark symbols represent light transmittance means within each cereal rye biomass level; and small light-colored symbols represent individual observations.
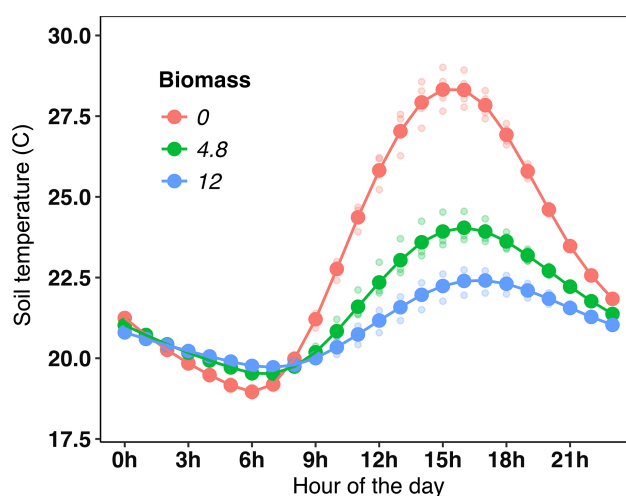


**Figure 9.** Hourly soil temperature (*C*) from 0- to 7.6-cm soil depth under the levels of cereal rye biomass of 0.0, 4.8, and 12.0 Mg ha$^{-1}$. Average 30-min interval readings collected from 0 to 70 d after establishment (DAE) in Brooklyn and Janesville, WI, in 2022 and 2023. Large dark symbols represent mean temperature; and small light-colored symbols represent the average of each replication across 4 site-years of data.
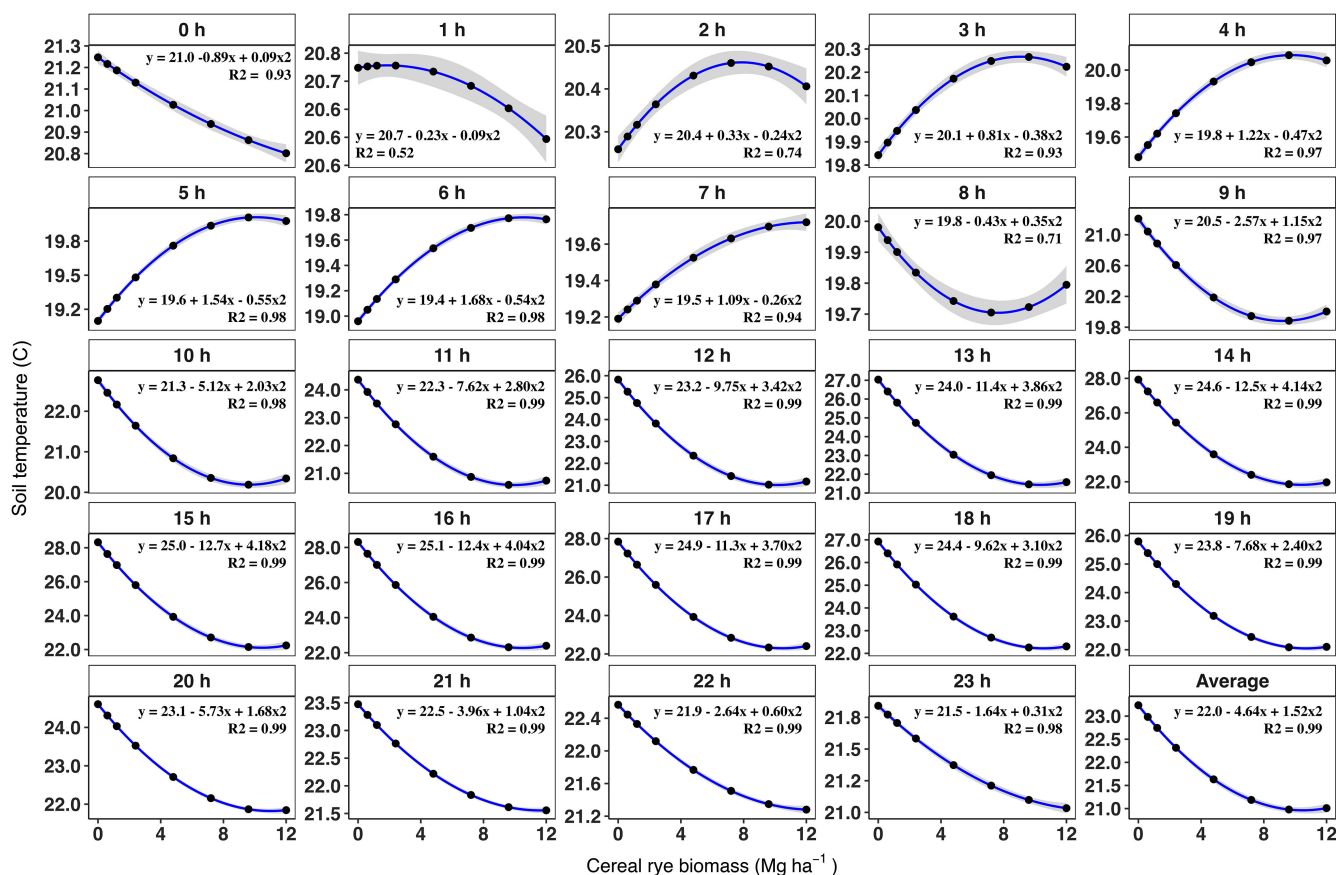
**Figure 10.** Effect of cereal rye biomass on soil temperature (C) at each hour of the day. Data estimated based on the mean temperature collected under the levels of cereal rye biomass of 0.0, 4.8, and 12.0 Mg ha$^{-1}$ in *Brooklyn and Janesville, WI, in 2022 and 2023*. Note that the y-axis is set to vary freely for each hour of the day. Further information on regression parameters can be found as Supplementary Table S3. Lines represent the model fit, points soil temperature means within each cereal rye dose, and shaded area around the lines the standard error.

germination and other problematic *Amaranthus* species, such as *A. retroflexus* and Palmer amaranth (*Amaranthus palmeri* S. Watson). Thus, the response of *A. tuberculatus* germination to the increase in mean temperature and temperature alternation supports the hypothesis that the reduction in soil temperature by the cereal rye biomass can be associated with the mechanism of suppression for this weed species. Moreover, because dry cereal rye biomass does not affect light quality (Teasdale and Mohler 1993) and *A. tuberculatus* germination can respond to the R:FR ratio (Leon and Owen 2003), it is likely that the effect of biomass on light transmittance only lowers the soil temperature but does not affect *A. tuberculatus* germination directly. Therefore, we can infer that the mechanism of suppression by the cereal rye biomass is regulated by the thermal effect that the biomass provides when blocking the sunlight, which consequently lowers the soil temperature and temperature amplitude. It should also be noted that lower light transmittance can affect the growth of new *A. tuberculatus* seedlings emerging through the cereal rye biomass. As Teasdale and Mohler (2000) reported, small-seeded weed species are more sensitive to high levels of cover crop biomass due to light deprivation and lower energy reserves. Although this effect can decrease the density of established seedlings and contribute to overall *A. tuberculatus* suppression, it is unlikely to affect seed germination directly. It is also important to emphasize that the effect of cereal rye biomass on light transmittance and soil temperature can vary over time with biomass decomposition. As

the cereal rye biomass decays, it is expected that its effect on soil microclimate will change and *A. tuberculatus* suppression will decline due to changes in effects on germination cues and decreased mechanical impedance by the residue over time.

Our results support that cereal rye can effectively suppress *A. tuberculatus* emergence and development and that the suppression level depends on the quantity and quality of cereal rye biomass. On average, 5.2 Mg ha$^{-1}$ of cereal rye biomass was required to reduce *A. tuberculatus* density by 50%. Such an amount can be used as a target for farmers adopting cereal rye to adapt their management practices to accumulate enough biomass and achieve effective *A. tuberculatus* suppression. Conversely, under low levels of cereal rye biomass ($\leq$2.38 Mg ha$^{-1}$), an increase in *A. tuberculatus* germination and density can be expected, especially under dry weather conditions after cereal rye termination. Thus, the cereal rye cover crop should be effectively established in the fall and terminated within an appropriate window in the following spring to ensure that adequate levels of biomass are produced before termination. As well as reducing *A. tuberculatus* density, the increase in cereal rye biomass ($\geq$7.2 Mg ha$^{-1}$) also delayed the onset of *A. tuberculatus* emergence, which is another benefit of this practice. The suppression provided by the cereal rye cover crop is likely driven by the lower soil temperature and temperature amplitude underneath the biomass. Future research is warranted to investigate the long-term impact of a cereal rye cover crop on *A. tuberculatus* seedbanks. It is unknown

for how long *A. tuberculatus* seeds can remain viable in the soil seedbank underneath the cereal rye biomass. Additionally, the role of potential allelopathic compounds released by cereal rye on *A. tuberculatus* suppression and the effect of cereal rye on nitrogen dynamics in the soil on *A. tuberculatus* recruitment are research areas to be further explored.

## References

Arsenijevic N, DeWerff R, Conley S, Ruark M, Werle R (2022) Growth and development of multiple waterhemp (*Amaranthus tuberculatus*) cohorts in corn and soybeans. Front Agron 4:1037483

Bish M, Dintelmann B, Oseland E, Vaughn J, Bradley K (2021) Effects of cereal rye seeding rate on waterhemp (*Amaranthus tuberculatus*) emergence and soybean growth and yield. Weed Technol 35:838–844

Boyd NS, Van Acker RC (2003) The effects of depth and fluctuating soil moisture on the emergence of eight annual and six perennial plant species. Weed Sci 51:725–730

Burgos NR, Talbert RE (2000) Differential activity of allelochemicals from *Secale cereale* in seedling bioassays. Weed Sci 48:302–310

Camargo Silva G, Bagavathiannan M (2023) Mechanisms of weed suppression by cereal rye cover crop: a review. Agron J 115:1571–1585

[CTIC/SARE/ASTA] Conservation Technology Information Center, Sustainable Agriculture Research and Education, and American Seed Trade Association (2023) National Cover Crop Survey Report 2022–2023. https://www.sare.org/wp-content/uploads/2022-2023-National-Cover-Crop-Survey-Report.pdf. Accessed: January 2, 2024

Cornelius CD, Bradley KW (2017) Influence of various cover crop species on winter and summer annual weed emergence in soybean. Weed Technol 31:503–513

Duke SO, Powles SB (2008) Glyphosate: a once-in-a-century herbicide. Pest Manag Sci 64:319–325

Faleco F, Oliveira M, Arneson N, Renz M, Stoltenberg D, Werle R (2022) Multiple herbicide resistance in waterhemp (*Amaranthus tuberculatus*) accessions from Wisconsin. Weed Technol 36:597–608

Finney DM, White CM, Kaye JP (2016) Biomass production and carbon/nitrogen ratio influence ecosystem services from cover crop mixtures. Agron J 108:39–52

Green JM (2014) Current state of herbicides in herbicide-resistant crops. Pest Manag Sci 70:1351–1357

Grint KR, Arneson N, Oliveira MC, Smith DH, Werle R (2022) Cereal rye cover crop terminated at crop planting reduces early-season weed density and biomass in Wisconsin corn-soybean production. Agrosystems Geosci Environ 5:e20245

Heap I (2023) The International Herbicide-Resistant Weed Database. www.weedscience.org Accessed: September 6, 2023

Hodgskiss CL, Young BG, Armstrong SD, Johnson WG (2022) Utilizing cover crops for weed suppression within buffer areas of 2,4-D-resistant soybean. Weed Technol 36:118–129

Keshtkar E, Kudsk P, Mesgaran MB (2021) Perspective: common errors in dose–response analysis and how to avoid them. Pest Manag Sci 77:2599–2608

Leon RG, Knapp AD (2004) Effect of temperature on the germination of common waterhemp (*Amaranthus tuberculatus*), giant foxtail (*Setaria faberi*), and velvetleaf (*Abutilon theophrasti*). Weed Sci 52:67–73

Leon RG, Owen MDK (2003) Regulation of weed seed dormancy through light and temperature interactions. Weed Sci 51:752–758

Liebman M, Basche AD, Nguyen HTX, Weisberger DA (2022) How can cover crops contribute to weed management? A modelling approach illustrated with rye (*Secale cereale*) and *Amaranthus tuberculatus*. Weed Res 62:1–11

Mattson MP (2007) Hormesis defined. Ageing Res Rev 7:1–7

Mohler CL, Teasdale JR (1993) Response of weed emergence to rate of *Vicia villosa* Roth and *Secale cereale* L. residue. Weed Res 33:487–499

Nichols V, Martinez-Feria R, Weisberger D, Carlson S, Basso B, Basche A (2020) Cover crops and weed suppression in the U.S. Midwest: a meta-analysis and modeling study. Agric Environ Lett 5:e20022

Nunes J, Arneson N, DeWerff R, Ruark M, Conley S, Smith D, Werle R (2023a) Planting into a living cover crop alters preemergence herbicide dynamics and can reduce soybean yield. Weed Technol 37:226–235

Nunes J, Arneson N, Wallace J, Gage K, Miller E, Lancaster S, Mueller T, Werle R (2023b) Impact of cereal rye cover crop on the fate of preemergence herbicides flumioxazin and pyroxasulfone and control of *Amaranthus* spp. in soybean. Weed Sci 71:493–505

Osipitan OA, Dille JA, Assefa Y, Radicetti E, Ayeni A, Knezevic SZ (2019) Impact of cover crop management on level of weed suppression: a meta-analysis. Crop Sci 59:833–842

Peterson MA, Collavo A, Ovejero R, Shivrain V, Walsh MJ (2018) The challenge of herbicide resistance around the world: a current summary. Pest Manag Sci 74:2246–2259

Picapietra G, Acciaresi HA (2021) Junglerice (*Echinochloa colona* L.) seedling emergence model as a tool to optimize pre-emergent herbicide application. Ital J Agron 16:1845

R Core Team (2022) R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org

Reed HK, Karsten HD (2022) Does winter cereal rye seeding rate, termination time, and N rate impact no-till soybean? Agron J 114:1311–1323

Ritz C, Baty F, Streibig JC, Gerhard D (2015) Dose-response analysis using R. PLoS ONE 10(12):e0146021

Schramski JA, Sprague CL, Renner KA (2021) Effects of fall-planted cereal cover-crop termination time on glyphosate resistant horseweed (*Conyza canadensis*) suppression. Weed Technol 35:223–233

Schwartz L, Norsworthy JK, Young BG, Bradley KW, Kruger GR, Davis VM, Steckel LE, Walsh MJ (2016) Tall waterhemp (*Amaranthus tuberculatus*) and Palmer amaranth (*Amaranthus palmeri*) seed production and retention at soybean maturity. Weed Technol 30:284–290

Steckel LE (2007) The dioecious *Amaranthus* spp.: here to stay. Weed Technol 21:567–570

Steckel LE, Sprague CL, Stoller EW, Wax LM (2004) Temperature effects on germination of nine *Amaranthus* species. Weed Sci 52:217–221

Striegel S, Oliveira MC, DeWerff RP, Stoltenberg DE, Conley SP, Werle R (2021) Influence of postemergence dicamba/glyphosate timing and inclusion of acetochlor as a layered residual on weed control and soybean yield. Front Agron 3:788251

Sweeney AE, Renner KA, Laboski C, Davis A (2008) Effect of fertilizer nitrogen on weed emergence and growth. Weed Sci 56:714–721

Teasdale JR, Mohler CL (1993) Light transmittance, soil temperature, and soil moisture under residue of hairy vetch and rye. Agron J 85:673–680

Teasdale JR, Mohler CL (2000) The quantitative relationship between weed emergence and the physical properties of mulches. Weed Sci 48:385–392

Van Wychen L (2022) 2022 Survey of the Most Common and Troublesome Weeds in Broadleaf Crops, Fruits & Vegetables in the United States and Canada. Weed Science Society of America National Weed Survey Dataset. http://wssa.net/wp-content/uploads/2022 weed survey broadleaf crops.xlsx. Accessed: September 5, 2023

Weisberger DA, Bastos LM, Sykes VR, Basinger NT (2023) Do cover crops suppress weeds in the U.S. Southeast? A meta-analysis. Weed Sci 71:244–254

Werle R, Sandell LD, Buhler DD, Hartzler RG, Lindquist JL (2014) Predicting emergence of 23 summer annual weed species. Weed Sci 62:267–279

Westwood JH, Charudattan R, Duke SO, Fennimore SA, Marrone P, Slaughter DC, Swanton C, Zollinger R (2018) Weed management in 2050: perspectives on the future of weed science. Weed Sci 66:275–285

Wickham H, Averick M, Bryan J, Chang W, McGowan LD, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller E, Bache SM, Müller K, *et al.* (2019) Welcome to the tidyverse. J Open Source Softw 4:1686

Williams MM, Mortensen DA, Doran JW (1998) Assessment of weed and crop fitness in cover crop residues for integrated weed management. Weed Sci 46:595–603

Yadav R, Jha P, Hartzler R, Liebman M (2023) Multi-tactic strategies to manage herbicide-resistant waterhemp (*Amaranthus tuberculatus*) in corn–soybean rotations of the U.S. Midwest. Weed Sci 71:141–149

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14:415–421