Environmental Pollution 185 (2014) 295–306

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Herbicide impact on non-target plant reproduction: What are the toxicological and ecological implications?☆

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ARTICLE INFO

Article history:
Received 31 May 2013
Received in revised form
23 August 2013
Accepted 4 October 2013

Keywords:
Non-target plant reproduction
Herbicide effect
Ecological risk assessment
Ecotoxicological implications
Non-crop habitats

ABSTRACT

Declining plant diversity and abundance have been widely reported in agro-ecosystems of North America and Europe. Intensive use of herbicides within cropfields and the associated drift in adjacent habitats are partly responsible for this change. The objectives of this work were to quantify the phenological stages of non-target plants in in-situ field situations during herbicide spray and to compare plant susceptibility at different phenological stages. Results demonstrated that a large number of non-target plants had reached reproductive stages during herbicide spray events in woodlots and hedgerows, both in Canada and Denmark where vegetation varies considerably. In addition, delays in flowering and reduced seed production occurred widely on plants sprayed at the seedling stage or at later reproductive periods, with plants sprayed at reproductive stages often exhibiting more sensitivity than those sprayed as seedlings. Ecological risk assessments need to include reproductive endpoints.

1. Introduction

Fertilizers and herbicides are the most widely used chemicals in farmlands and have been instrumental in the tremendous increase in crop productivity since World War II (Boutin, 2013). However, there has also been growing concerns about declining plant species richness, abundance and diversity (Fried et al., 2009) both within cropfields and in adjacent habitats including field margins, hedgerows, ditches, as well as small woodlots and wetlands (Andreasen and Stryhn, 2008; Crone et al., 2009; Romero et al., 2008; Storkey et al., 2012; Sutcliffe and Kay, 2000). Many plant species associated with agroecosystems have become rare to the extent that they are registered in the Red Data Books (International Union for Conservation of Nature) of several countries, including several arable species considered agricultural weeds (Albrecht and Mattheis, 1998; Türe and Böcük, 2008; Wilson, 1994). Failure to adequately assess and properly regulate herbicide effects can have important ecological implications for plant survival, seed production, long-term seedbank replenishment and eventual species composition of not only primary producers, but also species at other trophic levels.

Although fertilizer use is of great concern (Kleijn and Verbeek, 2000), this paper will primarily address herbicide effects and assessment. Herbicides used in agriculture for weed control in major crops are primarily sprayed in May or June in Canada (as per pesticide labels http://pr-rp.hc-sc.gc.ca/ls-re/index-eng.php). In most European countries, herbicides are sprayed several times in any given year depending on the crops (Strandberg et al., 2012). In Denmark, spring sown crops are usually sprayed with herbicides in April and May while autumn sown crops are sprayed in September and October. In the Netherlands, an average of 5.7 herbicides are sprayed on food crops (between three and nine depending on the crops) and 10.3 (between six and 15) herbicides per year are applied on field cultivated flower crops (EFSA, 2012). Though it has not yet been quantified, it is likely that herbicides will reach weeds and non-target plants at all phenological stages depending on the application time.

When plants are sprayed in cropfields and sublethal doses of herbicides reach non-target plant species in adjacent habitats through drift, runoff and/or volatilisation, resultant effects on sensitive species can be observed in any of four ways: a) Plants at the seedling stage during spray will have their vegetative parts affected, b) the same plants could express the effect through negative impacts on seed production at later stages, c) plants at the

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http://dx.doi.org/10.1016/j.envpol.2013.10.009

reproductive phase during spray have their seed production impacted or d) the vegetative parts of the F1 generation are affected (Fig. 1). Therefore, it appears that seedlings and plant species at late vegetative and reproductive stages may be affected differently, and this is most likely influenced in turn by the type of herbicide applied.

For regulatory purposes, greenhouse tests utilizing species growing singly in pots or in monoculture are required to assess the potential undesirable effects of herbicides on non-target, wild plants found within the vicinity of croplands. These tests are performed on emerging seedlings or on plants at the 2–6 leaf stage (usually using crops as surrogates for wild species) with effects recorded 14–28 days after the spray event (OECD, 2006; USEPA, 2012 – Fig. 1a). Several greenhouse studies have effectively shown that the seedling stage was more sensitive to herbicides than later growth stages, at least for some species (Boutin et al., 2000; Zwerger and Pestemer, 2000). However, other studies have shown that some species that have reached the reproductive stage following exposure exhibited negative herbicidal effects at doses below those observed for the seedling or vegetative stages (Boutin et al., 2000; Carpenter and Boutin, 2010; Carpenter et al., 2013; Riemens et al., 2008, 2009; Strandberg et al., 2012). In some cases, reproductive endpoints (seed production or measurable equivalent) may be more appropriate to assess than aboveground vegetative biomass, for instance when plants are exposed at later developmental stages when growth has ceased (Steadman et al., 2006; Strandberg et al., 2012; Walker and Oliver, 2008). The ISO protocol (2005; Fig. 1b) was developed to examine both the inhibition of growth and the reproductive capacity of plants following soil contamination (not specific to pesticides) under controlled conditions using two test species: a rapid-cycling variant of turnip rape (*Brassica rapa* CrGC syn. Rbr) and oat (*Avena sativa* L.). Though this test assesses both the vegetative and reproductive effects of contaminants on plants, it is not usually conducted for pesticide registration. There is no known protocol to determine effects when plants are sprayed at maturity (see Fig. 1c, d).

The objectives of this work were multi-faceted and aimed to address some of the above-mentioned issues. Our first objective was to quantify the numbers and types of species present at the vegetative and reproductive stage in non-crop habitats during herbicide spray events to indicate potential risks to non-crop plant reproduction. Results from two experiments with woodlots (Canada) and hedgerows (Denmark) are presented. Our second objective was to measure effects of herbicides on the initiation of flowering. This information was obtained from four field and greenhouse studies conducted in Canada and Denmark. Our third objective was to compare endpoints (vegetative and reproductive) and phenological stages at spray with the purpose of developing a more realistic estimation of effects which could be used in risk assessment. To meet this third objective, new and existing data in the published literature were compiled and analysed.

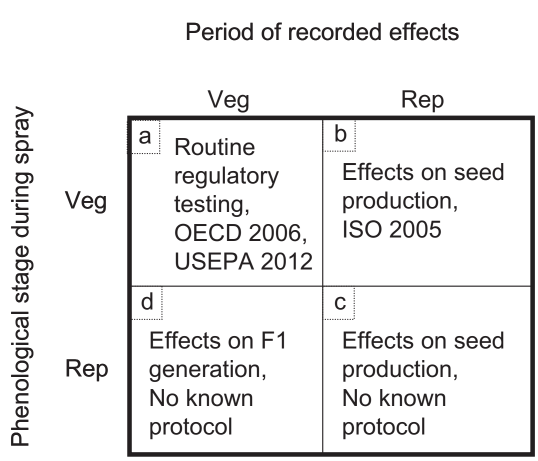

Fig. 1. Representation of phenological stage at spray (or testing) time and stage of recorded herbicide effects. Veg = Vegetative, Rep = Reproductive period. Letters within quadrants are used for reference purposes in the text. OECD (Organisation for Economic Co-operation and Development) 2006, USEPA (United States Environmental Protection Agency) 2012 and ISO (International Organization for Standardization) 2005 refer to standard guidelines for plant toxicity testing.

2. Material and methods

This article presents work from seven experimental studies, including unpublished experiments as well as unpublished data that were part of experiments already published.

2.1. Vegetation/Phenology surveys

Two experiments were conducted to determine plant phenology at the time of herbicide spraying. In EXP1, three woodlots (S1–S3) were selected in south-western Ontario, Canada (480971E–4757140N). The S1 woodlot was fairly open and dry and had been grazed a few years prior to the study (only surveyed in 1993). The S2 woodlot sustained some disturbance due to a cabin access path. It contained a wide mix of spring ephemeral vegetation typical of rich, well-drained soils. The S3 woodlot was characterised by a small stream and ponds. The main tree species found in the three woodlots included iron wood (*Ostrya virginiana* (Mill.) K. Koch), bitternut hickory (*Carya cordiformis* (Wangenh.) K. Koch), sugar maple (*Acer saccharum* Marshall), blue beech (*Carpinus caroliniana* Walter), American beech (*Fagus grandifolia* Ehrh.) and silver maple (*Acer saccharinum* L.).

The woodlots were adjacent to three different fields, all planted with soybean (*Glycine max* (L.) Merr.) in 1993, corn (*Zea mays* L.) in 1994 and wheat (*Triticum aestivum* L.) and corn in 1996. Herbicides (imazethapyr in 1993, dicamba in 1994 and MCPA in 1996) were sprayed under normal operational conditions by the farmer in May of each year (no trial was conducted in 1995). Herbicide application occurred in the early morning or evening, when no precipitation was forecasted; wind speed was at 8 km/h or less in the direction of the woodlot from across the planted field.

Quadrats were established along ten transects (at 10 m distances) per woodlot positioned perpendicular to the field. Five transects were abutted to an in-field 15 m buffer zone where no spray occurred and five transects had no buffer zone. Each transect consisted of 1 m^2 quadrats placed at 1, 2, 4, 8, 16 and 32 m distances into the woodlots. All vegetation below 2 m in height was surveyed for species composition. The phenological stage (vegetative or flowering) was recorded prior to the spray operation while symptoms of herbicidal impact (comparing qualitative visual assessment prior to and after the spray) were recorded four times between May and July. As long as one plant of a given species in a quadrat was flowering the species was considered at the reproductive stage for that quadrat.

In EXP2, 40 hedgerows were surveyed in Denmark in organic and conventional farming systems. In the first trial, starting in 2007, ten hedgerows on conventional farms were surveyed for three years. In the second trial in 2008, a further ten hedgerows were selected in conventional farms along with 20 hedgerows in organic sites, and were surveyed for four years (UTM coordinates: 517126E to 594023E–6168669N to 6259952N). Hedgerows were selected as pairs of organic and conventional to eliminate landscape effects and were similar in terms of woody species composition, management, orientation, age (80–150 years old) and crops on the neighbouring fields (cereals). All hedgerows (at least 400 m in length) had one to three rows of deciduous trees and shrubs along the entire length and a 0.5–1 m wide zone covered with herbaceous species at the field's edge. The main hedgerow trees and shrubs were oneseed hawthorn (*Crataegus monogyna* Jacq.), sweet cherry (*Prunus avium* (L.) L.), European mountain-ash (*Sorbus aucuparia* L.), Swedish whitebean (*S. intermedia* (Ehrh.) Pers.), European alder (*Alnus glutinosa* (L.) Gaertn.) and dwarf honeysuckle (*Lonicera xylosteum* L.).

Sampling of the flowering ground flora was performed in the herbaceous zone on the west-facing side. In each hedgerow sampling was conducted within fifteen 0.5 by 0.5 m permanent quadrats placed on a 100 m transect with a distance of 6.5 m between successive quadrats. Sampling was carried out monthly from May to mid-September. A survey of herbicide usage near ten hedgerows examined from 2007 to 2009 confirmed that applications occurred from the end of March until late October, with few treatments happening in June and July. At each sampling day the phenological stage of all vascular plants found within each quadrat was recorded. Any given species was recorded as flowering in a given month (May–September) if it was in flower in a specific hedgerow in any year, meaning that the maximum count for each species for a given month is twenty for organic and conventional farming types.

EXP2 data were also used to assess delays in flowering in relation to herbicide usage. The total number of species was tabulated for overall phenological assessment, and a list of 57 species preferably used by pollinators in Denmark was built based on expert knowledge and a thorough literature review (Benton, 2006;