Wine Australia for Australian Wine

Grapevine responses to injuries caused by different herbicides - Can we visually identify different sorts of herbicide injury in grapevines based on foliage and fruit symptoms?



FINAL REPORT FOR INCUBATOR PROJECT

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Project summary:

Herbicide drift can have devastating consequences on grapevines and their developing crop. The source and type of herbicide causing the damage is, however, often difficult to identify, partially because the visual symptoms that develop after exposure to a specific herbicide have not yet been fully described in grapevines. The aims of this study were to (1) chronicle the symptoms of 2,4-D, Dicamba, MCPA and glyphosate drift on grapevines, (2) investigate their impacts on leaf functioning, berry development and composition, and (3) assess carry-over effects on vine productivity in the following season. Simulated herbicide drifts were applied to potted vines at the cessation of flowering and the progression of symptom development was monitored until fruit maturity. While the four herbicides induced similar visual symptoms, there were some unique features that could allow their differentiation. Dicamba exposure exhibited upward leaf rolling in conjunction with yellow and brown interveinal lesion development. Unlike MCPA, 2,4-D damage did not exhibit leaf margin necrosis, whereas severely deformed lateral shoot leaves developed only after 2,4-D exposure. Glyphosate related injuries emerged later than those induced by other herbicides. Photosynthesis of newly emerged mature leaves was impaired by prior 2,4-D, MCPA and glyphosate exposure. Fruit yield was significantly reduced by 2,4-D, mainly as a result of bunch necrosis. This herbicide also increased juice titratable acidity but most of the other basic compositional parameters were not affected. While root structural development was limited by 2,4-D, Dicamba and MCPA, none of the herbicides altered root carbohydrate reserve levels. Primary bud necrosis was pronounced especially following 2,4-D and Dicamba exposure. Therefore, 2,4-D presents a significant hazard not only on the yield of the current season, but also for the next.

Aims and background:

Grapevines are sensitive to those herbicides used widely against invasive weeds in agricultural regions. Broadacre crop farmland, roadsides, and lawns in proximity to vineyards are regularly treated with different herbicides or combinations thereof. Herbicide spray particles, especially those of the phenoxyacetic acid group, can drift several kilometres off-target (Felsot et al., 2011).

The grapevine injuries caused by different types of herbicides, however, are often visually similar. The phenoxyacetic acid herbicides [such as 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA)] function as synthetic auxins, and are renowned for causing widespread damage to grapevines. 3,6-Dichloro-2-methoxybenzoic acid (Dicamba), another commonly-used growth-regulating herbicide, also functions as an auxin. Auxinic herbicides ultimately cause uncontrolled cell division in developing tissues, and grapevine injury signs may resemble leaf deformations, reduced fruit set and delayed ripening (Al-Khatib et al., 1993; Bondada et al., 2011). Glyphosate is readily used on the vineyard floor to control weeds between vines. Exposure to glyphosate blocks aromatic amino acid synthesis (tyrosine, tryptophan and phenylalanine) in affected tissues, therefore inhibiting plant metabolism related to the shikimate pathway (Cobb and Raede, 2011).

Glyphosate injuries can induce distorted leaf shapes, flower abortion and reduced berry skin colour intensity in grapevines (Al-Khatib et al., 1993; Donnini et al., 2016).

Only a limited number of studies have addressed the consequences of herbicides on grapevine performance (physiology and productivity) despite an ever-increasing use of herbicides world-wide. The first objective of this research was to characterise the shoot, foliage and fruit symptoms induced by 2,4-D, MCPA, Dicamba or glyphosate and to identify any unique features that will facilitate the identification of a specific herbicide following exposure. The second objective was to elucidate the repercussions of herbicide exposure on vine functioning and physiology. The third objective was to assess how herbicide exposure may impact on the vine's health and reproductive capacity in the following season. More specifically, the following main aims were included in the research:

1. To describe the visual leaf, shoot and fruit injuries as induced by each herbicide.
2. To assess the consequences of herbicide exposure on leaf functioning including photosynthesis.
3. To identify repercussions on fruit yield and composition, and vine biomass including structural roots.
4. To evaluate if herbicide exposure impinges on long-term vine carbohydrate reserve stores.
5. To determine which herbicides induce tissue necrosis within the buds.

The information provided can help identify injury symptoms linked to specific herbicides. Moreover, the research improves our understanding of seasonal and inter-seasonal responses to specific herbicides.

Materials and methods:

Experimental design:

Forty-eight, five-year-old potted (50 L) Tempranillo grapevines were used in this study. Vines were shoot-thinned before flowering so that each comprised 11 fruiting shoots (between 15 and 18 bunches per vine). Three randomly selected grapevines were dismantled into leaves, fruit and roots at the termination of flowering (E-L stage 26; Coombe and Dry, 2004) and stored for further analysis. These vines represented the population of grapevines before treatment (henceforth referred to as Harvest 1). The remaining grapevines were allocated to a single herbicide treatment or non-sprayed control. Each treatment vine was individually sprayed with the pre-allocated herbicide in an automated laboratory-sized boom sprayer. The sprayer allowed delivery of precise volume of herbicide to each vine, with simulated herbicide drifts of 65 g/ha effectively applied. This rate represents approximately 7, 12, 10 and 10% of the recommended labelled rates for 2,4-D, Dicamba, MCPA and glyphosate, respectively. Furthermore, these rates align with strong but realistic off-target herbicide drift movement (Bode, 1987; Maybank et al., 1978). The spray applications were allowed to dry overnight, and vines were subsequently placed in a randomised block design within an outdoor birdproof enclosure. The experimental design allowed for three destructive harvest dates, and three replicates per treatment or

control in addition to the harvest date. The destructive harvests were conducted when berries were pepper-corn sized (E-L 31), beginning to soften (E-L 34) and at berry maturity (E-L 38). These dates are henceforth referred to as Harvests 2, 3 and 4, respectively. The periods between the harvests are henceforth referred to as Intervals 1 (between the cessation of flowering and the peppercorn stage), 2 (between the peppercorn stage and véraison) and 3 (between véraison and berry maturity).

Measurements, and sample and data collection:

Detailed images and visual references were collected every other day to document foliar, shoot and fruit injury responses to each known herbicide as the experiment progressed.

Fortnightly leaf gas exchange measurements were undertaken under saturating light between 11:00 and 13:00 on bright, sunny days. A 6400 Portable Photosynthesis Analyser (LiCor, Nebraska, USA) was used at a PPFD of 2000 $\mu mol.m^{-2}.s^{-1}$, with a reference gas CO_2 concentration of 410 ppm and temperature set to the expected average air temperature during measurement. Two older leaves (directly opposite a bunch) and two younger fully expanded leaves (within the top five shoot nodes) per vine were used to measure photosynthesis and stomatal conductance during each measurement day. The younger leaves were already formed by treatment implementation, and typically displayed visual signs of herbicide injury after treatment, whereas bunch zone leaves did not visually appear injured (Appendix 2). The same leaves were used to measure chlorophyll index using a Spad meter (Spad-502, Minolta Corp Ltd., Osaka, Japan). The Spad measurement per leaf consisted of an average of three readings.

Fifteen pre-selected grapevines (three for each herbicide treatment and for the control) were dismantled on the destructive harvest dates. Whole root systems, leaves and all fruit were collected from each vine. The fresh weights of these organs were determined, and the roots and leaves were subsequently dried at 60 °C to determine the weights after drying. During the destructive harvests, root subsamples were collected, consisting of full-length root parts taken from within 10cm from the basal part of the trunk, between 2 and 6mm in diameter. Soil particles were shaken off and the roots rapidly rinsed with deionized water, prior to freezing in liquid N.

One hundred berries per vine were used to determine fresh weight per berry and juiced to measure total soluble solid concentration (°Brix). The juice pH and titratable acidity were determined by sodium hydroxide (0.1 M) titration using an automatic titrator (Metrohm Fully Automated 59 Place Titrando System, Metrohm AG, Herisau, Switzerland) to an end point of pH 8.2. The ammonium and α-amino acid concentrations of the juice were determined by using a commercially available enzymatic assay kit, designed for an Arena discrete analyser (Thermofisher, Scoresby, Australia). The yeast assimilable N (YAN) concentration was subsequently calculated from the ammonium and free amino N (FAN) (Iland et al. 2004). Fruit total anthocyanin concentration was analysed from a 50-berry subsample per vine. Whole berries were homogenised (Ultra-Turrax T25, IKA), and the total anthocyanin concentration determined as described by Iland et al. (2004). The number of berries per vine was estimated by dividing the total fresh fruit weight per vine by the average berry fresh weight per vine.

Frozen root subsamples were ground to a fine powder under liquid N (A11 basic analytical mill, IKA, Selangor, Malaysia) and freeze-dried (Gamma 1-16 LSC, Christ, Osterade am Harz, Germany). Starch and total soluble sugar concentrations in a 20-mg dried sample of ground tissue were determined following the methods outlined in Rossouw et al. (2017). The total root non-structural carbohydrate content was expressed as the combination of root starch and total soluble sugars. Root structural biomass was calculated by subtracting non-structural carbohydrate content from the root dry weight.

The basal three nodes of five shoots per vine (with the most basal bud being a non-count bud), in addition to one whole shoot per vine, were collected during the destructive harvest to evaluate the presence of bud necrosis. The compound buds were dissected and observed under a stereo microscope (Leica MZ8, Leico microsystems, Wetzlar, Germany). Records were taken of any signs of necrotic tissues being present within compound buds, as sampled for each harvest date (Figs. S4 and S5, Appendix 4). Buds were evaluated as containing visible necrotic tissues or not. Additionally, incidents of full primary bud necrosis were recorded for the final sampling date only (Fig. S6, Appendix 4).

Statistical analysis

Data were analysed using Statistica 13 (Tibco, Palo Alto, CA, USA), with the analysis of variance (ANOVA) used to test the significance of each variable. Fisher's least significant difference (LSD) test was used to identify significant differences between means ($P < 0.05$).

Results and discussion:

Visual herbicide injury symptoms:

Appendix 2 provides images and a full description of the development of the obvious visual shoot, foliar and fruit injuries that were triggered by the different herbicides as the season progressed. In addition, the initiation of key visible shoot (Fig. S1), leaf (Fig. S2) and fruit (Fig. S3) injury signs after exposure to each of the four different herbicides, is illustrated in timelines in Appendix 3.

Leaf functioning:

Consequences of grapevine exposure to the four herbicides on leaf appearance and morphology are discussed in Appendix 2. However, more than a visual assessment was required to conclude if leaf functioning was significantly affected by exposure to the herbicides. The leaf blade chlorophyll index (CI), stomatal conductance (g_s) and photosynthetic rates (A) were quantified for each interval during the experiment.

During the present study, control vine bunch zone leaves did not exhibit higher CI values than those of the herbicide treated vines (Fig. S7, Appendix 5). However, CI values of younger control vine leaves were higher than those of all herbicide treatments during ripening (Interval 3) (Fig. S7, Appendix 5). Chlorophyll index is measured to non-destructively estimate the concentration of chlorophyll in the leaf blade, and can inform on the overall leaf photosynthetic potential and physiological status of the plant (Steele et al., 2008). Younger leaves in particular have a significant role in canopy photoassimilation during the berry ripening period when the older leaf photosynthetic capacity has already been impaired

(Poni et al., 1994). Exposure to all herbicides limited the extent to which CI values increased between the pre- and post-véraison period of the current study. The inferior young leaf CI during the post-véraison period, as induced by 2,4-D, Dicamba, MCPA or glyphosate, was thereby to some degree detrimental to canopy photoassimilation capacity during fruit ripening.

The g_s values of bunch zone leaves were not significantly affected by any treatment (Fig. S8, Appendix 5). Likewise, g_s of younger leaves did not differ among treatments between flowering and véraison (Fig. S8, Appendix 5). During ripening (Interval 3), young leaves of control vines and those treated with Dicamba, exhibited greater g_s compared to 2,4-D or glyphosate treatments. Exposure to 2,4-D and glyphosate thus restricted the gas exchange capacity of younger leaves during berry ripening.

Photosynthetic rates of bunch zone leaves were not significantly affected by any herbicide (Fig. 1A). However, younger leaves of control vines exhibited higher *A* than those treated with MCPA, between flowering and berry maturity (Intervals 1 and 3), and additionally higher rates than those treated with 2,4-D and glyphosate from véraison (Interval 3) (Fig. 1B). Young leaves of Dicamba treated vines exhibited higher *A* than those treated with 2,4-D, MCPA and glyphosate during ripening (Interval 3). Exposure to 2,4-D, MCPA and glyphosate inhibited young leaf photosynthesis during berry ripening.

Differences in leaf photosynthesis among treatments were driven by g_s variation. Exposure to 2,4-D, MCPA and glyphosate suppressed new leaf g_s during berry ripening, thereby limiting the photosynthetic capacity of these leaves during a period of considerable fruit carbohydrate sink demand. Impairment of g_s following 2,4-D exposure has previously been described by Bondada (2011a), however, the results of the present study additionally demonstrate the inhibitory effect of 2,4-D, MCPA and glyphosate on younger leaf *A*. Bondada (2011b) described stomatal abnormalities, including collapsed and cracked guard cells, developing after grapevine 2,4-D injury. These abnormalities would perturb photosynthesis, and likely explain the impaired photosynthesis found for 2,4-D injured leaves during the present study. Further work is needed to also determine the implications of Dicamba, MCPA and glyphosate on the grapevine leaf stomatal structure, however, MCPA and glyphosate also impaired younger leaf stomatal functioning, whereas Dicamba exposure was not significantly detrimental.

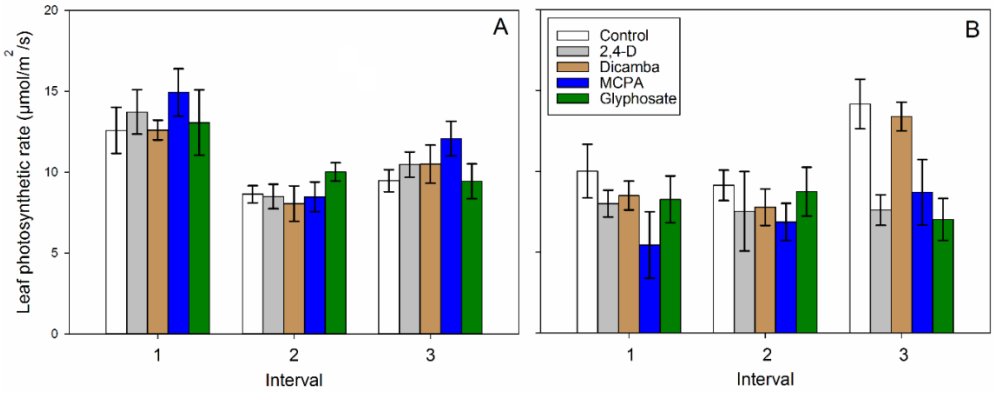


Fig. 1: Impact of herbicide exposure at the cessation of flowering on bunch zone (A) and apical leaf (B) photosynthetic rates (*A*) during Intervals 1 (post-flowering to peppercorn sized berries), 2 (peppercorn sized berries to véraison) and 3 (véraison to fruit maturity) (mean ± SE).

Leaf biomass and fruit yield

The total leaf dry weight accumulation per vine was measured to quantify leaf biomass development during the experiment. Leaf dry weights increased significantly for all vines, however, control vines had greater leaf dry weights compared to those treated with 2,4-D and MCPA by the peppercorn stage (Harvest 2), and additionally higher values than those treated with 2,4-D and Dicamba, by véraison (Harvest 3) and full maturity (Harvest 4), respectively. Glyphosate treated vines had greater leaf dry weights than those treated with Dicamba or MCPA, by the final harvest. Therefore, Dicamba exposed vines exhibited lower leaf biomass than both control vines and those treated with glyphosate by fruit maturity.

As illustrated in Appendix 2, Dicamba was particularly injurious to apical shoots, which progressed basipetally as the experiment progressed. Shoot necrosis was also initiated by 2,4-D and MCPA exposure, but not to the same extent as that caused by Dicamba, at least by visual assessment. The inferior leaf biomass of Dicamba-exposed vines by the time fruit were fully mature, suggests that the Dicamba treated vines had reduced vine photosynthesis during the berry ripening period due to lower canopy leaf area.

When the fruit were fully mature only 2,4-D treated vines had significantly lower yield than those of the control (Fig. 2B and Table 1). As detailed in Appendix 2, 2,4-D exposure caused prominent berry abortion and bunch necrosis during the experiment. These observations agree with the reduced fruit yield that was apparent in the 2,4-D treated vines at fruit maturity. Injuries related to 2,4-D exposure are, therefore, particularly detrimental to grapevine fruit yield.

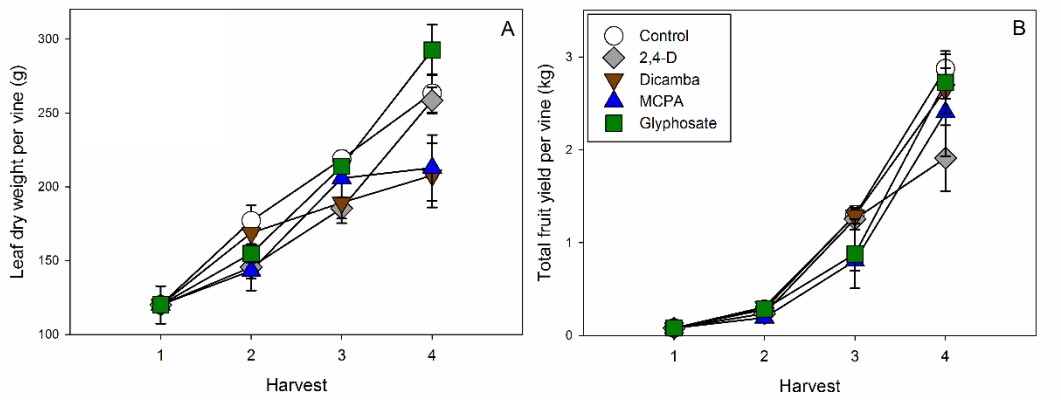


Fig. 2: Impact of herbicide exposure on leaf dry weight (A) and fruit yield development per vine (B) (mean ± SE).

Mature fruit characteristics and composition

The number of berries per 2,4-D treated vine was significantly lower to that of the control and Dicamba treated vines by the final harvest (Table 1). In fact, 2,4-D exposure caused a 41% reduction in vine berry number relative to the control. This result clearly illustrates the extent to which the 2,4-D induced bunch necrosis reduced vine crop load by the time berries were fully mature.

The average fresh weight per berry of glyphosate treated vines was greater than that of the control by the time fruit were fully mature (Table 1). This larger berry size can likely be attributed to the slight

reduction in berry numbers following glyphosate exposure ($P > 0.05$). In contrast, Dicamba vines exhibited significantly reduced berry fresh weights relative to the control. As illustrated in Appendix 2, Dicamba exposure induced significant millerandage that extended over the length of the bunch. The average fresh weight of mature berries was thereby effectively reduced by Dicamba.

The only measured berry compositional parameter significantly affected by any herbicide treatment at flowering, was juice titratable acidity (TA). 2,4-D exposure resulted in higher TA relative to the control. Others have found that grapevine exposure to 2,4-D can, to some degree, cause uneven or delayed fruit ripening (Inaba et al., 1974; Ogg et al., 1991). As highlighted earlier in our visual assessment, 2,4-D treatment resulted in uneven berry ripening, with many green ('unripe' appearing) berries still present within the bunch by the final harvest. Grape TA after 2,4-D exposure is not consistent across seasons, however, and may be dependent on the developmental stage when the exposure occurred (Ogg et al., 1991). While the increased juice TA after 2,4-D exposure needs further investigation to clarify the underlying factors, none of the herbicides used in the present study had significant effects on juice total soluble solid concentration, pH, yeast assimilable nitrogen (YAN) concentration or berry anthocyanin concentration by the final harvest (Table 1).

Table 1 Consequences of herbicide exposure (2,4-D, Dicamba, MCPA, glyphosate) on fruit yield per vine, estimated berry number per vine, average berry fresh weight, and juice titratable acidity, pH, yeast assimilable nitrogen concentration, and berry anthocyanin concentration on the final harvest date (mean ± SE).

Treatment	Yield (kg/vine)	Berries per vine	Average berry weight (g)	TSS (°Brix)	pH	TA (g/L)	YAN (mg/L)	Berry anthocyanin concentration (mg/g fwt)
Control	**A**[1] 2.9 ± 0.2	**A** 1512 ± 59	**B** 1.90 ± 0.03	23.3 ± 0.3	4.24 ± 0.03	**B** 3.89 ± 0.01	118.7 ±6.5	0.90 ± 0.04
2,4-D	**B** 1.9 ± 0.4	**B** 896 ± 246	**AB** 2.09 ± 0.08	22.7 ± 0.5	4.21 ± 0.02	**A** 4.28 ± 0.04	146.6 ± 15.1	0.83 ± 0.05
Dicamba	2.7 ± 0.1	**A** 1590 ± 64	**C** 1.67 ± 0.04	23.0 ± 0.4	4.24 ± 0.03	4.08 ± 0.11	149.8 ± 15.5	0.74 ± 0.09
MCPA	2.4 ± 0.5	1200 ± 219	**AB** 2.00 ± 0.09	22.1 ± 0.3	4.22 ± 0.03	4.17 ± 0.16	138.1 ± 5.0	0.80 ± 0.05
Glyphosate	2.7 ± 0.3	1254 ± 124	**A** 2.17 ± 0.09	23.0 ± 0.5	4.21 ± 0.04	3.94 ± 0.21	137.9 ± 31.8	0.79 ± 0.07

[1]significant differences between the treatments are indicated as uppercase letters.

Root structural development and carbohydrate reserves:

The root structural dry weight increased for all treatments between flowering and fruit maturity (Fig. 3A). However, by the final harvest, control vine root structural biomass was superior to those of vines exposed to 2,4-D, Dicamba and MCPA, whereas glyphosate treated vines exhibited superior values than the MCPA treatment. These results therefore indicate that 2,4-D, Dicamba and MCPA exposure inhibited root structural growth to some degree. Exposure to auxin-type herbicides can induce supra-

optimal concentrations of auxin in the affected plant, and in turn, ethylene is produced which causes a relative inhibition of tissue growth (Cobb and Raede, 2011). The systemic nature of these herbicides means that their active constituents are translocated to non-contact tissues such as the roots, where it may subsequently affect growth. Further work is, however, needed to fully understand the implications of 2,4-D, Dicamba and MCPA on not only grapevine root growth but also their functioning.

Root total non-structural carbohydrate (NSC) content increased to some degree for all treatments between flowering and fruit maturity (Fig. 3B). No significant differences were, however, found in root NSC content, on a per vine basis, among treatments at any stage. In terms of root total NSC concentration, significant differences among treatments were only observed at the peppercorn stage (Harvest 2) (Fig. S9, Appendix 5). At this period, control vines exhibited greater values than those of the 2,4-D, Dicamba and MCPA treatments, whereas the glyphosate treatment had higher values than vines treated with MCPA. However, no significant differences in root NSC concentration occurred by full berry maturity (Harvest 4). The impairment of young leaf photoassimilation induced by 2,4-D, MCPA and glyphosate exposure, therefore, did not significantly limit root carbohydrate reserve replenishment at fruit maturity. The carbohydrate reserves are an important energy source for newly developing canopies and roots in spring and maybe also contribute to fruit set and inflorescence size. These results indicate that, under the conditions of this study, vine prepardness for the following season, in terms of an existing energy source, was thus not significantly hampered by the herbicide drift exposure in the previous spring.

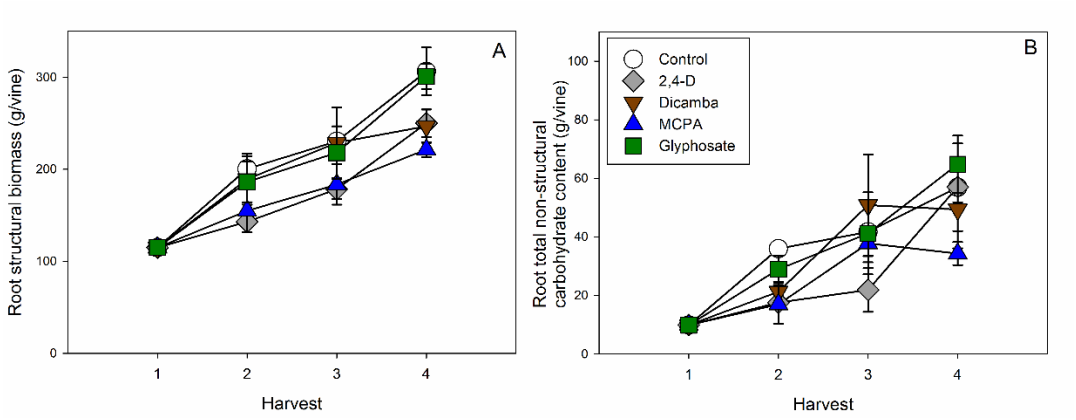


Fig. 3: Impact of herbicide exposure on root structural biomass (A) and total non-structural carbohydrate accumulation per vine (B) (mean ± SE).

Bud necrosis:

The consequence of herbicide exposure on bud health was assessed by quantifying the percentage of buds along the shoot containing visual necrotic tissues. There was no effect of herbicide exposure at Harvest 2, however by véraison (Harvest 3), vines exposed to 2,4-D and MCPA exhibited a higher percentage of basal buds (buds 2 and 3 from the shoot base) containing necrotic tissues compared to the control (Fig. 4A). The fruitfulness of these buds is essential for spur pruned vines as they are retained and subsequently develop into shoots the following season (with the most basal ‘non-count’ bud usually

not developing). By Harvest 4, all herbicide treated vines exhibited a higher percentage of basal buds presenting necrotic tissues relative to the control.

In addition to basal buds, the buds on nodes four to eight from the shoot base (buds 4-8) are usually retained with cane pruning. By the véraison stage, 2,4-D exposed vines presented a significantly higher percentage of buds at nodes 4-8 containing necrotic tissues compared to the control (Fig. 4B). By the final harvest (at fruit maturity), both 2,4-D and Dicamba treated vines presented a higher percentage of buds at nodes 4-8 containing necrotic tissues relative to the control. Likewise, those buds located on nodes 9 and upward contained necrotic tissues after exposure to both 2,4-D and Dicamba, as assessed at the final harvest (Fig. S10, Appendix 5). These results therefore indicate that all four herbicides were detrimental to the health of buds usually retained after spur pruning, whereas 2,4-D and Dicamba were also particularly detrimental to the health of those compound buds higher up the shoot.

By the final harvest, an elevated percentage of buds on nodes 2 and 3 of 2,4-D exposed vines exhibited primary bud necrosis (PBN) (Fig. 4C). Buds located on nodes 4-8 of 2,4-D and Dicamba treated vines exhibited a higher percentage of PBN than the control and glyphosate treated vines at the same stage. The younger buds located on nodes 9 and upwards of 2,4-D and Dicamba treated vines presented a higher percentage PBN than the control, MCPA and glyphosate treated vines by the final harvest. When PBN occurs, the primary bud within the compound bud is destroyed, and fewer fruitful buds may subsequently develop into shoots the next season, therefore reducing fruit yield considerably (Perez and Kliewer, 1990). Ogg et al. (1991) described the residual effect of 2,4-D injury on grape yield the season following exposure, where the number of bunches per vine as well as bunch weights were reduced. The increased incidence of PBN as induced by 2,4-D exposure during the current study, likely explains the reduced yields observed in Ogg's study. In summary, the current study demonstrates that through increased PBN, 2,4-D exposure has the potential to dramatically decrease the following season's yield when vines are spur pruned, and even more so when vines are to be cane pruned. Likewise, Dicamba induced PBN can be particularly detrimental to the following season's yield when vines are to be cane pruned.

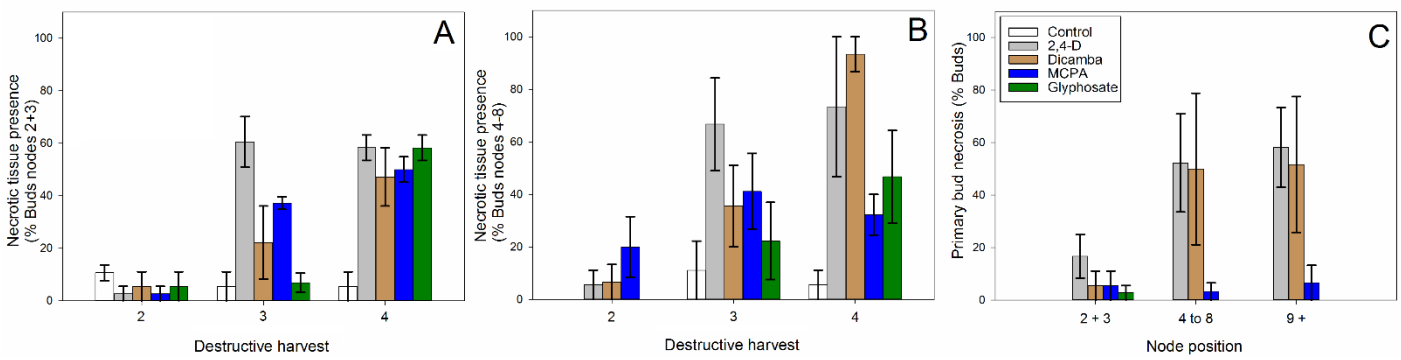


Fig. 4: Impact of herbicide exposure on percentage basal buds (A) and buds on nodes 4 to 8 (B) containing necrotic tissues by Harvests 1, 2 and 3, in addition to percentage buds on node positions 2+3, 4-9 and 9+ presenting primary bud necrosis by fruit maturity (C).

Conclusion and recommendations

A visual description of the morphological responses to 2,4-D, Dicamba, MCPA or glyphosate, as provided in this study, will assist grapegrowers to assess herbicide injuries and subsequently better manage the damage and identify the drift source. However, it remains clear that visual-only assessments can cause confusion, as many symptoms are not herbicide specific. This study has revealed that herbicides can have physiological consequences on grapevine functioning. 2,4-D, MCPA and glyphosate depressed younger leaf photosynthesis, which can be particularly detrimental to canopy functioning during fruit ripening. Exposure to 2,4-D can also be especially damaging to fruit yield due to an increased risk of bunch necrosis. The auxin-type herbicides inhibited root structural development, however, root carbohydrate reserve replenishment was not hampered by the herbicides. Importantly, 2,4-D and Dicamba was particularly harmful to grapevine bud health, and exposure to these herbicides may induce primary bud necrosis which may limit yield potential for the following season. Overall, this study produced novel results which will benefit the wine industry in terms of understanding how grapevines respond to herbicide damage, and the short- and longer-term implications thereof towards sustainable viticulture. Future work can include a comparison of drift rates and herbicide exposure levels at different growth stages, while additional herbicides such as diuron and oryzalin could also be assessed. Repercussions of two or more herbicide combinations could additionally be studied, as it is common practise to use herbicide combinations to combat problematic weeds in agricultural regions. The results provided here are for cv. Tempranillo only and widening these investigations to include other cultivars may reveal genotypic differences in the extent of herbicide drift damage.

Extension

- An article detailing the project background and aims, in addition to relevant literature information, was published in the Wine and Viticulture Journal as an introduction to the experiment (*Wine & Viticulture Journal Publication 2017*).
- Images and a description of the visual shoot, foliar and fruit injuries that were triggered by the different herbicides will be published as an extension article in the 2018/19 NSW DPI Grapevine Management Guide (Appendix 2).
- Results from this study were presented to the industry partner, grapegrowers and winemakers during the NSW DPI Pruning Workshop program in the Hunter Valley and Orange in June 2018.

Researcher benefit and feedback

Involvement in Wine Australia's Incubator initiative provided me with the opportunity to conduct applied research related to an important wine industry concern. This opportunity has strengthened my research skills, especially in terms of linking my research activity directly to practical viticulture. More flexibility in project start date would allow researchers to align their experimental design with the grapevine developmental cycles over the season, so that the specific industry issue can be properly addressed over the full 12-month period.

Appendix 1

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Appendix 2

Visual symptoms of herbicide drift on grapevine shoots, leaves and fruit

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Introduction

Herbicide drift towards non-target crops is unfortunately a common occurrence in agricultural regions. Grapevines can exhibit specific negative symptoms after exposure to most herbicides used to control weeds around broadacre crops, or next to roads and on lawns. Depending on climatic conditions, off-target drifts can move for several kilometres and can easily reach vineyards on neighbouring farms. Windy conditions, lower relative humidity and/or higher atmospheric temperatures are all factors contributing to the extent of injury from spray drift. However, linking specific symptoms to a particular herbicide can be difficult, making it problematic to identify the drift source and to avoid future incidents.

Some of the most widely used herbicides in Australian and global agriculture include 2,4-dichlorophenoxyacetic acid (2,4-D), 3,6-dichloro-2-methoxybenzoic acid (Dicamba), 2-methyl-4-chlorophenoxyacetic acid (MCPA) and glyphosate. Many plant growth-regulating herbicides, such as 2,4-D, Dicamba and MCPA, are renowned for causing drift issues. Phenoxyacetic acid type herbicides, including 2,4-D and MCPA, are particularly damaging to grapevines. Closer to home, glyphosate is commonly used in vineyards to control weeds between vines and can therefore easily reach off-target grapevines.

A simulated drift experiment on potted grapevines was recently conducted at the NWGIC in Wagga Wagga to better characterise grapevine injury symptoms to specific herbicides. Spring exposures (mid-November) to 2,4-D, Dicamba, MCPA and glyphosate were observed visually over several weeks on five-year-old Tempranillo grapevines at the cessation of flowering. An automated cabinet boom sprayer was used to apply rates of 65 g/ha A.I. of each herbicide to the allocated vines. This rate represents drifts between 7 and 12% of the recommended label rates of the herbicides. The onset of véraison occurred around 20 December 2017, while berry maturity was attained by 30 January 2018. The information below provides a description and images of the development of the obvious visual shoot, foliar and fruit injuries that were triggered by the different herbicides as the season progressed.

1. Shoot injuries

Herbicide exposure generally caused downward bending of apical shoot components and also entire shoots soon after 2,4-D, Dicamba or MCPA treatment. Glyphosate exposure, however, resulted in milder shoot injuries. Shoot tip necrosis (death, as evidenced by tissue browning and desiccation) was also obvious as the experiment progressed, and was induced to some degree by all four herbicides. Exposure to 2,4-D, Dicamba and MCPA induced shoot necrosis, in a basipetal direction from the tip over time. Shoot necrosis was particularly severe following Dicamba exposure. Below is a more specific description of the damage caused by each herbicide over time.

The first visible response to **2,4-D** was extensive downward bending (drooping) of the top 10-20 cm of the shoot tip from the day after treatment (Fig. 1A). These shoots continued to lose their turgor and wilt during the first three days following exposure. After about three days, shoot tips started curling, presenting a pig's tail appearance, and also becoming necrotic. Additionally, there was necrosis of the tendrils located near the shoot tips. Downward bending of more shoots and necrosis of additional shoot tips and tendrils continued during the 2nd week after 2,4-D application. By week four, shoot necrosis progressed basipetally, and considerable senescence (2-5 nodes) was observed

by week five (Fig. 1B). This was not universal, however, with some shoots instead exhibiting a zig-zag growth pattern with short internodes from this period (Fig. 1C). Lateral shoot development initiated within the sixth week after 2,4-D treatment, and continued until berry maturation, however, these were stunted and tended to crowd around the primary shoot (Fig. 1D).

Dicamba exposure induced the downward bending of shoot tips, clearly visible from the day after spray application (Fig. 2A). Leaf petioles also drooped down within the first week after treatment, giving these shoots a wilted appearance (Fig. 2A). Curling of shoot tips, in the shape of a pig's tail, occurred within the second week after Dicamba exposure (Fig. 2B), whereas necrosis of the shoot tips and tendrils initiated during the same period, and was particularly widespread from the third week after treatment. This was followed with the senescence of the top of the shoot (2-5 nodes) by week four (Fig. 2C), while shoot necrosis progressed basipetally for about 2-4 more nodes throughout weeks five to seven (Fig. 2D). A few lateral shoots emerged during the last two weeks of the experiment, however these appeared normal.

The shoots of grapevines sprayed with **MCPA** also bent down and resembled a wilted appearance from the day after treatment (Fig. 3A). Tendril and shoot tip necrosis, in addition to more severe shoot bending, occurred during the second week after MCPA exposure (Fig. 3B). Basipetal progression of shoot necrosis was obvious during week four, continuing for a few weeks (Fig. 3C). Normal appearing lateral shoot development initiated from week eight and continued towards berry maturity.

Downward bending of some shoots (approximately 5-10%) occurred from around four days after **glyphosate** exposure, while only minor shoot tip necrosis also emerged during the same time. Necrosis of a few additional shoot tips and tendrils continued during the second and third weeks after glyphosate treatment. Normal lateral shoot development became evident from the eighth week after treatment.

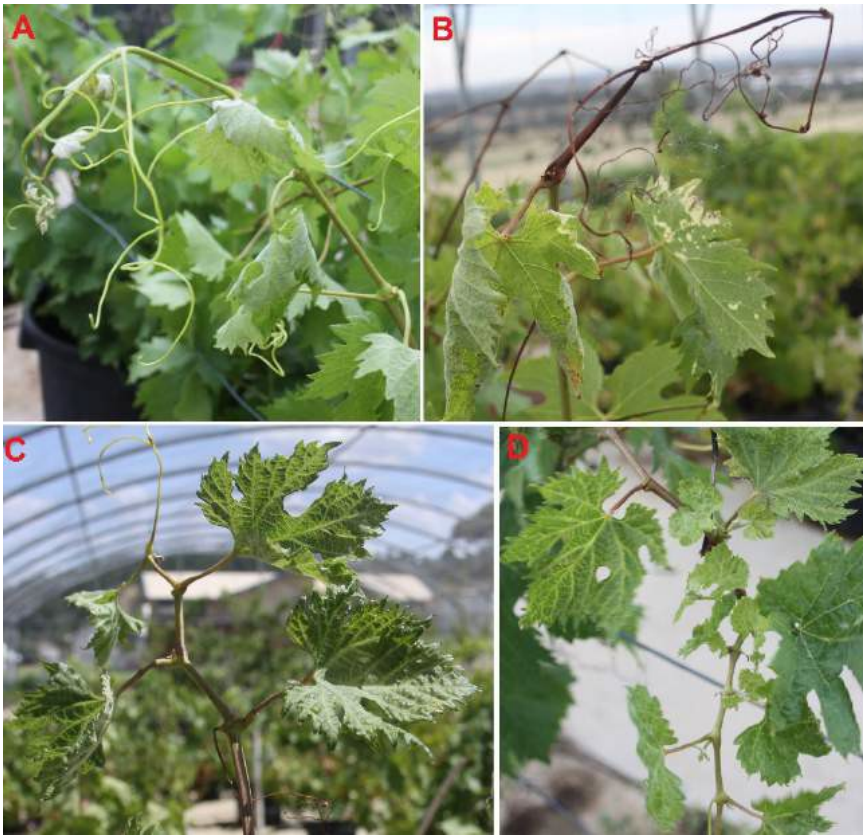


Fig. 1: Grapevine shoot appearance after **2,4-D** exposure. **A**: Downward bending of shoots the day after exposure. **B**: Shoot necrosis, and leaf upward folding and interveinal white chlorotic lesion development, at 29 days after exposure. **C**: Shoot

growth exhibiting a zig-zag pattern, while apical leaves appear uneven surfaced and cupped, 31 days after treatment. **D**: Stunted lateral shoots crowding around the primary shoot at 42 days after exposure.



Fig. 2: Grapevine shoot appearance after **Dicamba** exposure. **A**: Shoot tip drooping, and upward rolling of leaf blades in addition to downward bending of the petiole at two days after treatment. **B**: Shoot tip curling, and tip and tendril necrosis, 13 days after exposure. **C**: Shoot necrosis and tip senescence at 22 days after Dicamba application. **D**: Widespread downward bending of shoots and necrosis at 47 days after treatment.

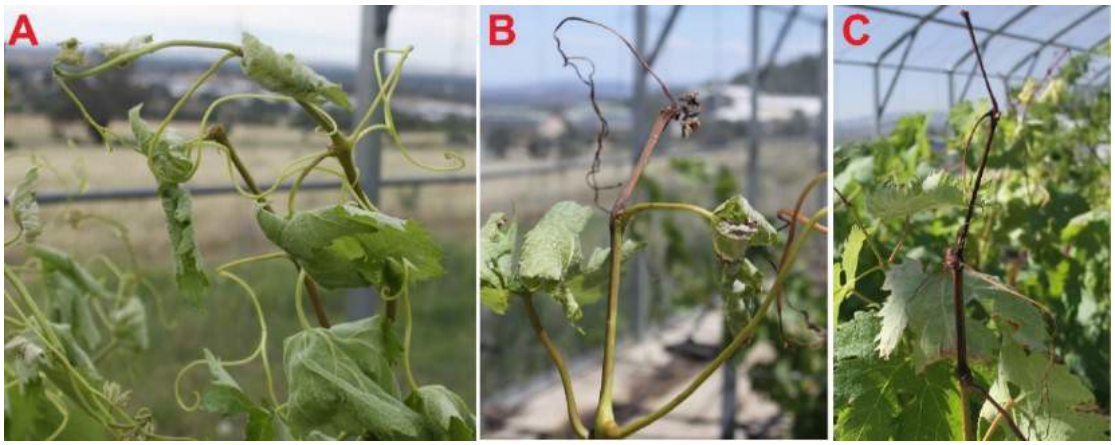


Fig. 3: Implications of **MCPA** exposure on grapevine shoot appearance. **A**: Downward bending of shoots, in addition to severe upward leaf blade rolling at two days after exposure. **B**: Shoot tip and tendril necrosis, as well as leaf blade upward rolling and margin necrosis at 15 days after treatment. **C**: Shoot necrosis progressing downward at 22 days after exposure.

2. Leaf injuries

The timing and symptoms of the leaf injuries were not always herbicide specific. Perhaps most distinct, however, was Dicamba exposure which induced leaf blade rolling in the upward direction in conjunction with the development of yellow and brown interveinal lesions. The leaf injury symptoms of 2,4-D, MCPA or glyphosate exposure were not easily discerned from each other at times. However, 2,4-D exposed vines specifically developed severely deformed lateral shoot leaves, whereas leaf blade

margin necrosis was noticeable after MCPA or glyphosate exposure only. Emergence of injury signs linked to glyphosate exposure was often delayed, while glyphosate exposure distinctly induced impaired apical (front) lobe development of young apical leaves.

Upward rolling of younger leaf blades was obvious within 24 hours after **2,4-D** exposure (Fig. 1A). After three days, many apical leaf blades appeared shrivelled and continued to roll inward to full leaf blade closure. The shrivelling of these leaf blades subsided slightly by the second week, however, upward cupping of most apical leaves was visible by this stage. Interveinal white chlorotic lesion development initiated on some of the cupped leaves by ten days after treatment. In contrast, young leaves near the top of the shoot exhibited a fan-shaped appearance from 12 days after exposure, with small cupped leaf blades, serrated margins (enations) and reduced interveinal spaces apparent (Fig. 4A). Prominent white interveinal lesions started to emerge on still expanding leaves a bit further down the shoots within the third week after spraying, in conjunction with mild upward leaf blade folding (Fig. 4B). Fan-shaped young leaves displayed thick and uneven, rutted blades, puckered spots and discolouration around leaf veins from the third week after treatment (Fig. 4C). By week five, many leaves on different shoot positions exhibited interveinal white or yellow chlorotic lesions and/or distorted blades (Fig. 4D). Severely deformed lateral shoot leaves emerged from week six, remaining small and lacking pigmentation to maintain a light green appearance (Fig. 4E). Lateral leaves were also very crowded around the shoot (Fig. 1E), and exhibited reduced or narrow interveinal spaces, crowded veins and sharp margin teeth from week seven (Fig. 4F).

Distinct upward margin rolling of apical leaves initiated from the day after **Dicamba** exposure (Fig. 5A), continuing throughout the first and second weeks after treatment. By two days after exposure, many fully closed leaf blades were visible on the youngest region of the shoot (Fig. 2A). Younger leaves still exhibited upward rolled margins by two weeks after exposure, as well as pale interveinal yellow lesions by this stage (Fig. 5B). Interveinal lesion development intensified from week three, with yellow and brown or black lesions appearing on many apical leaves in conjunction with upward leaf margin rolling (Fig. 5C). Development of leaf lesions continued throughout weeks four and five (Fig. 5D), and were present until the end of the experiment. Some leaves further down the shoots also started to exhibit upward margin rolling from week five onwards. Lateral shoot leaves emerged from week ten, presenting uneven surfaces and rounded blades.

Exposure to **MCPA** induced upward blade folding and epinasty of young apical leaves from the day after treatment (Fig. 6A). Two days after treatment, young leaf blades were severely rolled up to full closure (Fig. 3A), however, the tightness of leaf rolling was reduced by the second week (Fig. 6B). Young leaves on the uppermost two to three nodes appeared cupped by the second week after treatment, whereas the blades of expanding leaves further down the shoot continued to roll up. In the third week, leaf margin rolling continued on additional older expanding leaves (5-10 nodes below the shoot tip), whereas on apical leaves distinct white interveinal chlorotic lesions emerged along with margin necrosis (Fig. 6C). Deformation of young leaves continued during weeks four and five, resulting in uneven leaf surfaces and rough or sharp leaf margin serrations in addition to more severe margin necrosis (Figs. 6D and E). Upward rolling continued further down shoots during weeks six to nine, whereas additional young leaf margin necrosis development also occurred during this period. Lateral shoot development occurred from week eight, with these leaf blades formed in a round shape.

Apical leaf blades on the vines treated with **glyphosate** started to roll up from about four days after spraying, and margin necrosis also set in (Fig. 7A). Young leaf margin necrosis continued during the second and third weeks (Fig. 7B), along with the emergence of yellow or white interveinal chlorotic lesions on some of these leaves (Fig. 7C). Other types of young leaf blade distortion were evident from the fourth week, with the development of cupping, sharp margin serrations, crowded veins and uneven surfaces (Fig. 7D). Deformation of young leaves continued during week five, with the onset of distinctive white discolouration near the veins (Fig. 7E). By week six, some young leaf margins

appeared serrated, while impaired apical lobe development seemed to occur as the leaves expanded (Fig. 7F). More discolouration around young leaf veins was observed seven and eight weeks after treatment, when apical leaves also appeared fan-shaped with uneven surfaces and crowded veins. Leaf margin necrosis additionally progressed along the older nodes further down the shoot at around week eight. Lateral shoot leaves emerged from week nine, exhibiting leaf blades with little to no sinus differentiation.

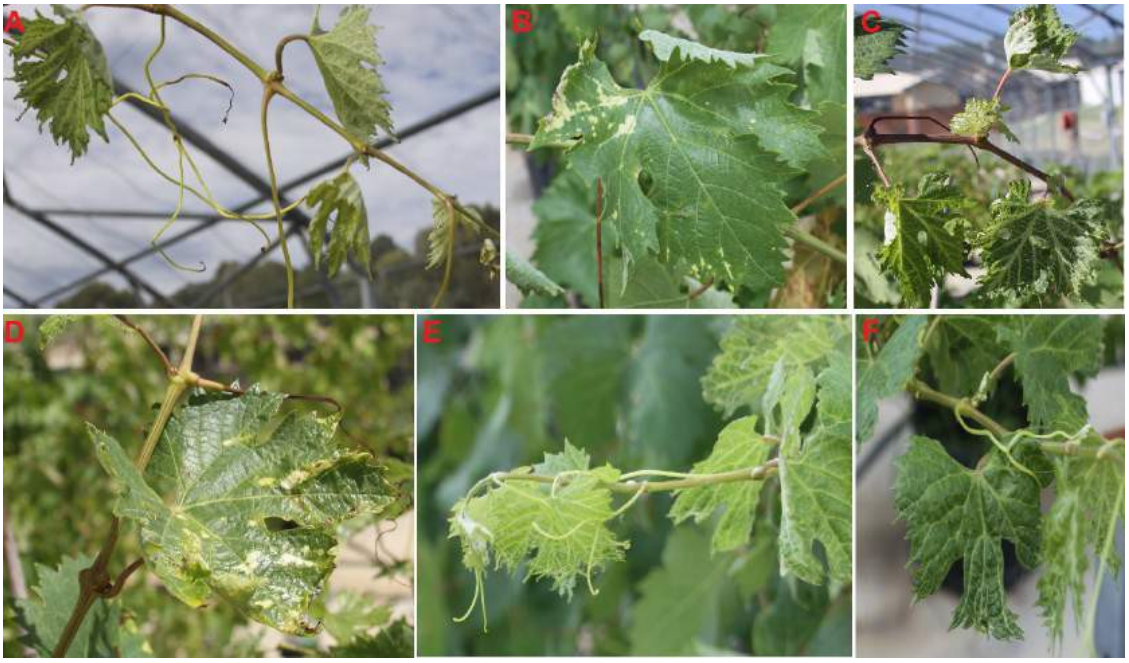


Fig. 4: Leaf appearance after **2,4-D** exposure. **A**: Fan-shaped apical leaf, exhibiting cupping, sharp margins and reduced interveinal spaces at 12 days after exposure. **B**: White interveinal lesion development and leaf margin upward folding at 18 days after treatment. **C**: Thick apical leaf blades with discolouration around veins and cupping at 50 days after exposure. **D**: Yellow interveinal lesions and distorted leaf blade shapes at 31 days after treatment. **E:** Severely deformed small, light coloured lateral shoot leaves at 42 days after treatment. **F**: Deformed lateral shoot leaves with crowded veins and narrow interveinal spaces at 44 days after treatment.

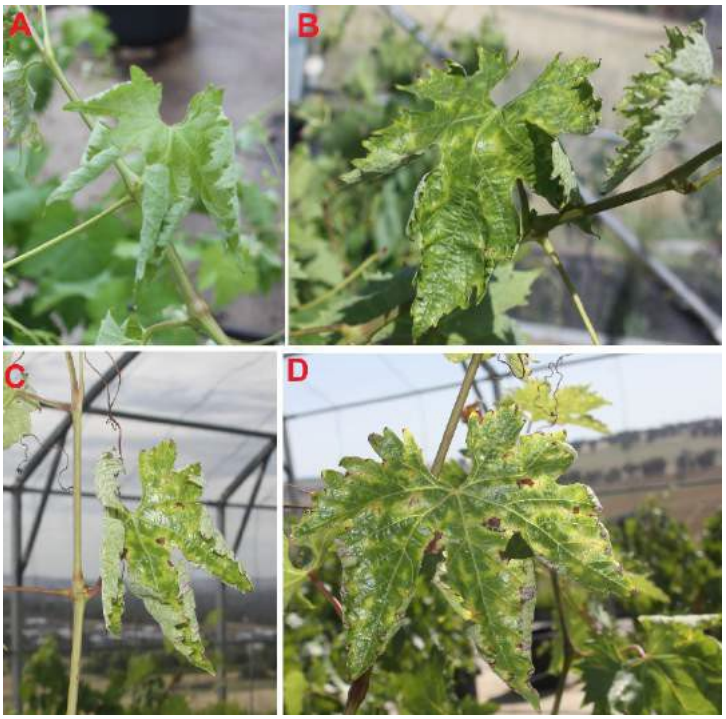


Fig. 5: Leaf appearance after **Dicamba** exposure. **A**: Upward margin folding the day after treatment. **B**: Minor upward margin rolling and pale yellow interveinal lesion development at 13 days after exposure. **C**: Yellow and brown interveinal lesions and upward leaf margin rolling at 19 days after treatment. **D**: Yellow and brown interveinal lesion and upward margin rolling at 32 days after exposure.

Appendix 2



Fig. 6: Leaf appearance after **MCPA** exposure. **A**: Upward blade folding and petiole epinasty one day after treatment. **B**: Upward folding and cupping of apical leaves at 13 days after exposure. **C:** White interveinal lesion development, and upward margin folding and necrosis at 18 days after treatment. **D**: Deformed apical leaves with uneven surfaces and white interveinal lesions at 26 days after treatment. **E**: Leaf margin necrosis and distorted blade shapes at 36 days after exposure.

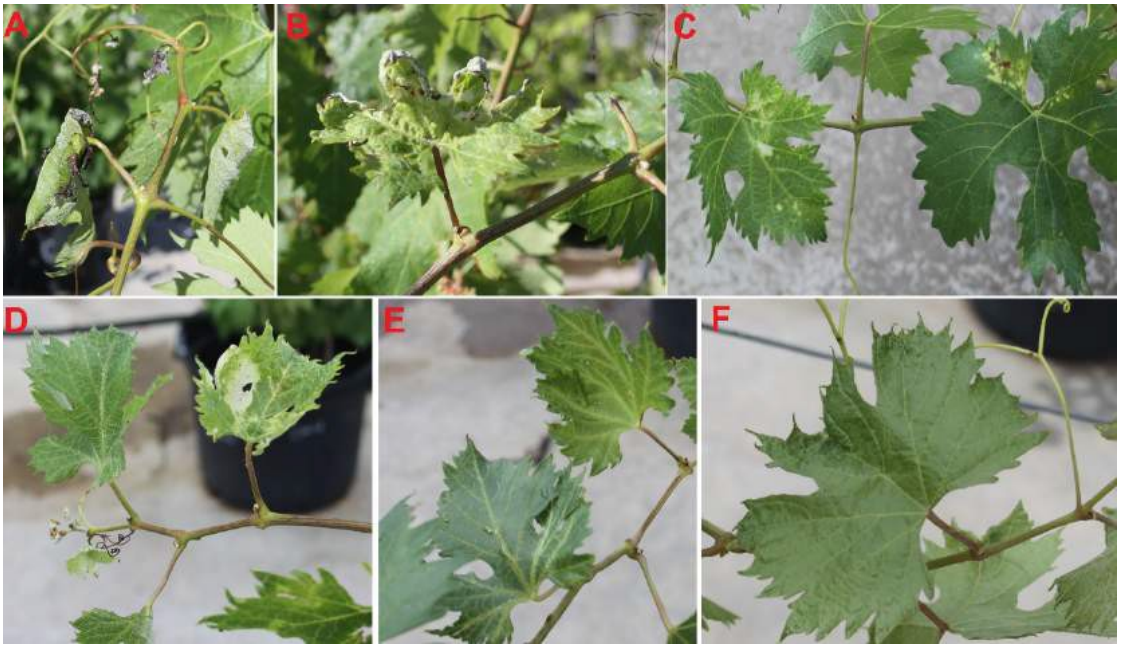


Fig. 7: Leaf appearance after **glyphosate** exposure. **A**: Upward leaf blade folding and margin necrosis occurring at four days after exposure. **B**: Margin necrosis at 22 days after treatment. **C**: White or yellow lesions developing on apical leaf blades at 16 days after exposure. **D**: Cupping of apical leaves, as well as uneven blade surfaces and white stain development at 29 days after exposure. **E**: Discolouration around leaf veins and crowding of veins apparent at 54 days after treatment. **F**: Impairment of young leaf apical lobe development at 58 days after exposure.

3. Fruit injuries:

Curving of bunch stems was the first and most prominent early bunch injury sign following exposure to 2,4-D, Dicamba or MCPA just after the cessation of flowering. Exposure to 2,4-D resulted

in the most severe visual symptoms, including noteworthy berry or whole bunch necrosis. Dicamba exposure was characterised by bunch millerandage ('hen and chicken' appearance), whereas glyphosate related bunch symptoms were mild and generally emerged later than those of the other treatments.

Minor bunch stem curvature was noticeable during the first week after **2,4-D** exposure, while necrosis or abortion of individual peppercorn sized berries and pedicels were also observed (Fig. 8A). By week three, fruit were pea-sized and more berry necrosis was evident. Full necrosis of some bunches or necrosis of the basal portion of the bunch, including the berries, pedicels and rachis were noteworthy by week five after treatment, just prior to the start of véraison (Fig. 8B). By week eight, when the fruit had intermediate sugar levels, ripening appeared uneven with some green berries still undergoing véraison. By week 11 at fruit maturity, various bunches still contained some green berries with berry necrosis widespread on many bunches.

Curvature of bunch stems was prominent within the first week after **Dicamba** application, with some minor berry abortion that was not evident in the control treatment. Most bunches had a 'hen and chicken' appearance from this period onwards (Fig. 9A). By week five, bunches of Dicamba treated vines exhibited distinct millerandage throughout the length of the bunch (Fig. 9B). A small number of whole bunches on Dicamba treated vines were necrotic by berry maturity.

Exposure to **MCPA** resulted in curved bunch stems and noteworthy millerandage a week after treatment (Fig. 10A). By week five, full necrosis of some bunches was noted, whereas bunch millerandage was still noticeable by the final harvest when the fruit was mature (Fig. 10B).

Glyphosate exposure only had minor effects on bunch appearance. However, mild bunch stem curvature was noted by the fifth week after treatment. Likewise, by week 11 when the fruit had matured, glyphosate treated vines exhibited mild millerandage.



Fig. 8: Bunch appearance after **2,4-D** exposure. **A**: Slight bunch stem curving, and necrosis of individual berries and pedicels eight days after treatment. **B**: Necrosis of whole bunches or basal bunch parts at 34 days after treatment.



Fig. 9: Bunch appearance after **Dicamba** exposure. **A**: Mild stem curvature and bunch millerandage at eight days after treatment application. **B**: Millerandage visible across the bunch length at 34 days after exposure.

Appendix 2



Fig. 10: Bunch appearance after **MCPA** exposure. **A**: Bunch stem curvature and bunch millerandage present at eight days after exposure. **B**: Bunch millerandage at 73 days after treatment.

Conclusions

The visual assessment and identification of grapevine damage related to 2,4-D. Dicamba, MCPA or glyphosate exposure can be confusing. Many injury signs caused by the each of the four herbicides are similar and practically hard to distinguish. However, Dicamba exposure induced unique injury signs, especially upon leaf development. Dicamba injured vines exhibited upward leaf rolling, in conjunction with yellow and brown interveinal lesion development. Being chemically similar, 2,4-D and MCPA exposure induced several comparable symptoms. However, unlike MCPA, 2,4-D damage did not exhibit leaf margin necrosis, whereas severely deformed lateral shoot leaves only developed after 2,4-D exposure. Glyphosate related injuries mainly emerged later than those induced by the other herbicides. Impaired development of the apical lobe of young leaf blades was perhaps the most distinct feature of glyphosate damage. This guide to visually identify grapevine responses to the above-mentioned herbicides can hopefully assist growers in future seasons to promptly recognize and address common herbicide drift related issues in vineyards. Not included in this report, the study also included an assessment of vine physiological and biochemical responses to the herbicides, which will provide further information useful to understand and address herbicide drift issues in vineyards.

What can I do to minimise damage after herbicide exposure?

- **Avoid or limit cane pruning**. Growth regulating herbicides (2,4-D, Dicamba and MCPA) impair bud fruitfulness, especially those on higher shoot positions.
- **Spur pruning is a safer option**. The basal bud health is less affected after exposure to growth regulating herbicides.
- **Avoid water stress during berry ripening**. Young leaf photosynthesis is impaired by phenoxyacetic acid herbicides and glyphosate, and irrigation practices can contribute to the retention and functioning of older leaves. Older leaf functioning subsequently becomes important towards fruit ripening, particularly if herbicide affected vines carry a substantial crop load.
- **Apply post-harvest or late season fertilisation and irrigation** especially to younger vines with developing root systems. Growth regulating herbicides impair root growth and stimulation of root development during the post-harvest/late season period becomes crucial. Avoiding water constraints and nutritional deficiencies during this period should promote the development of a healthier root system.

Appendix 3

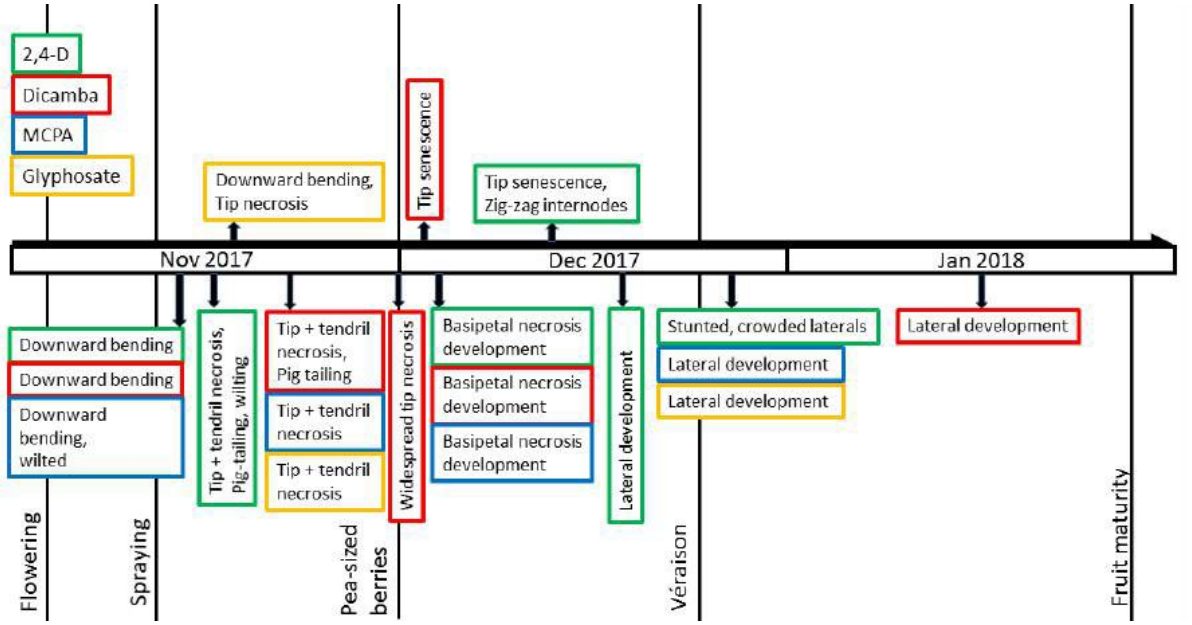


Fig. S1: Timeline of the visual emergence of the major shoot injury symptoms following grapevine exposure to either 2,4-D, Dicamba, MCPA or glyphosate.

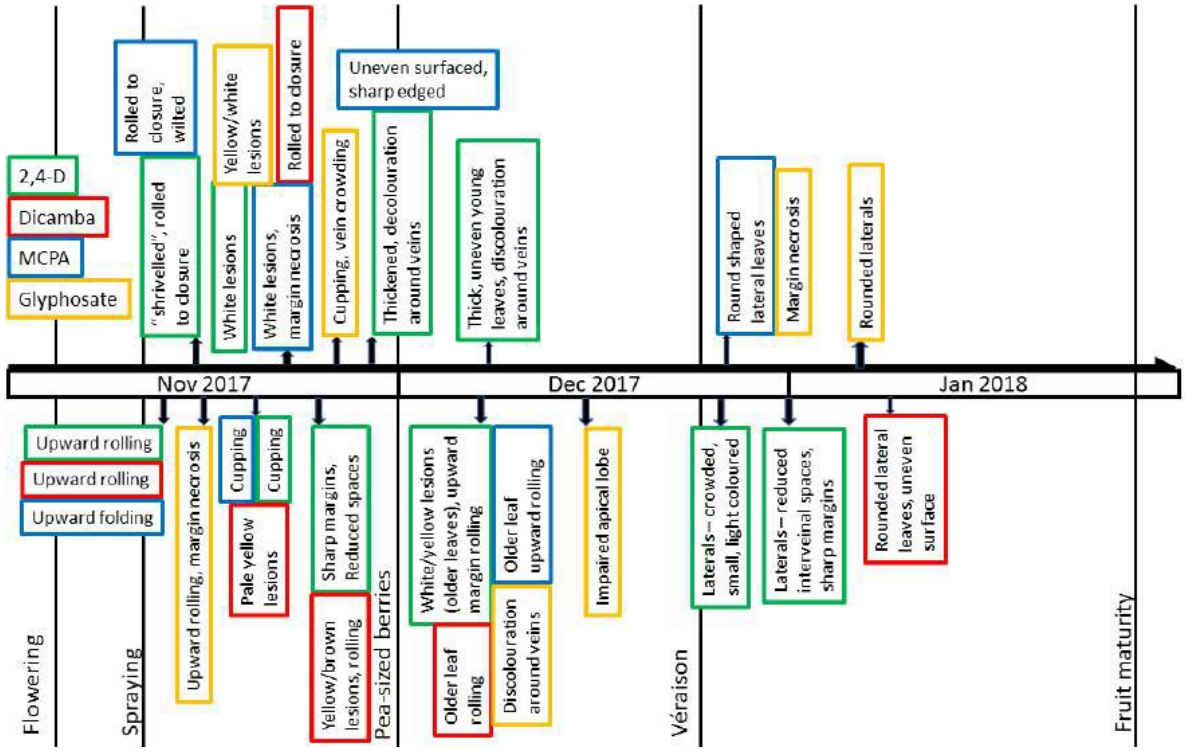


Fig. S2: Timeline of the visual emergence of major leaf injury signs following grapevine exposure to either 2,4-D, Dicamba, MCPA or glyphosate.

Appendix 3

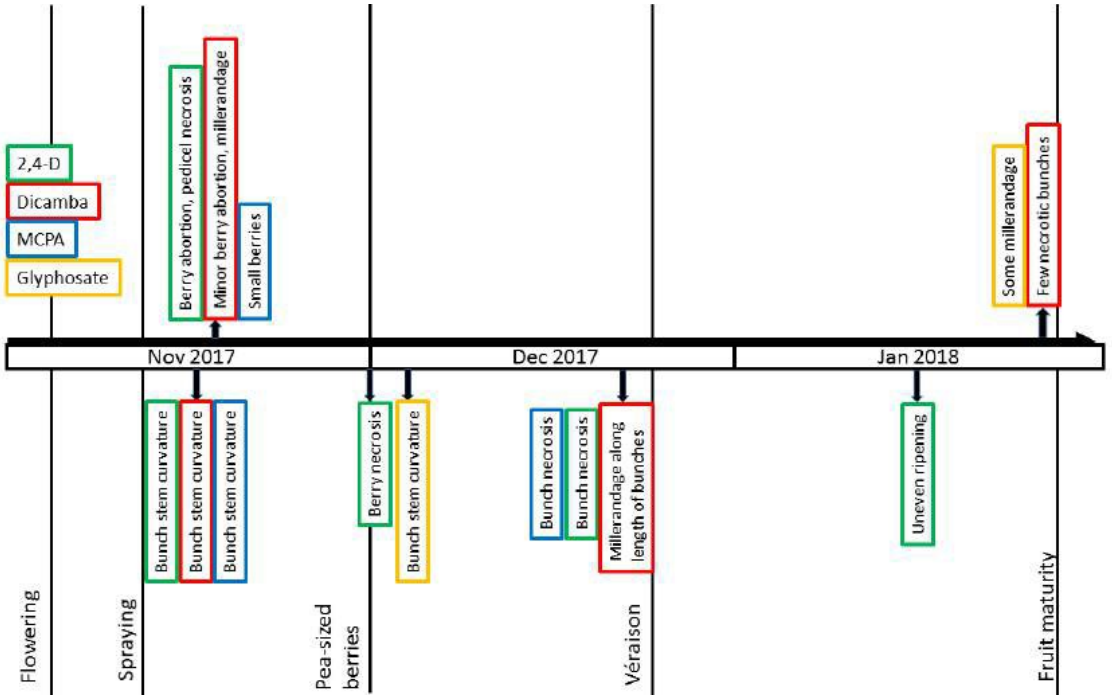


Fig. S3: Timeline of the visual emergence of major bunch injury signs following grapevine exposure to either 2,4-D, Dicamba, MCPA or glyphosate.

Appendix 4

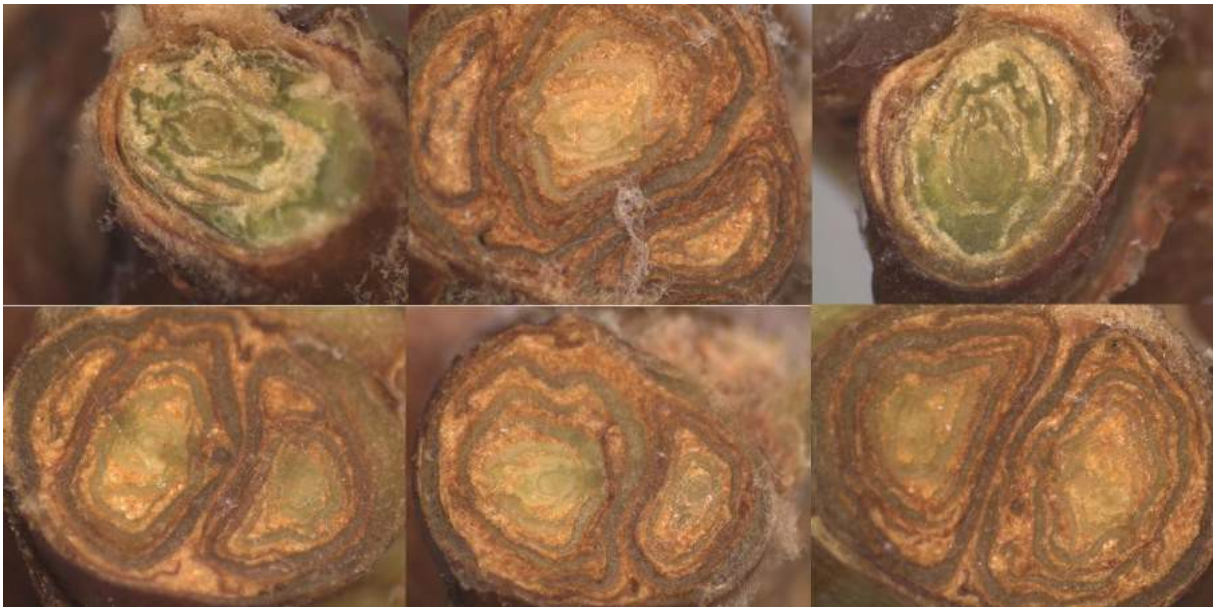


Fig. S4: Microscopic images of visually healthy compound buds sampled during the experiment.



Fig. S5: Microscopic images of compound buds sampled during the experiment, containing necrotic tissues.

Appendix 4

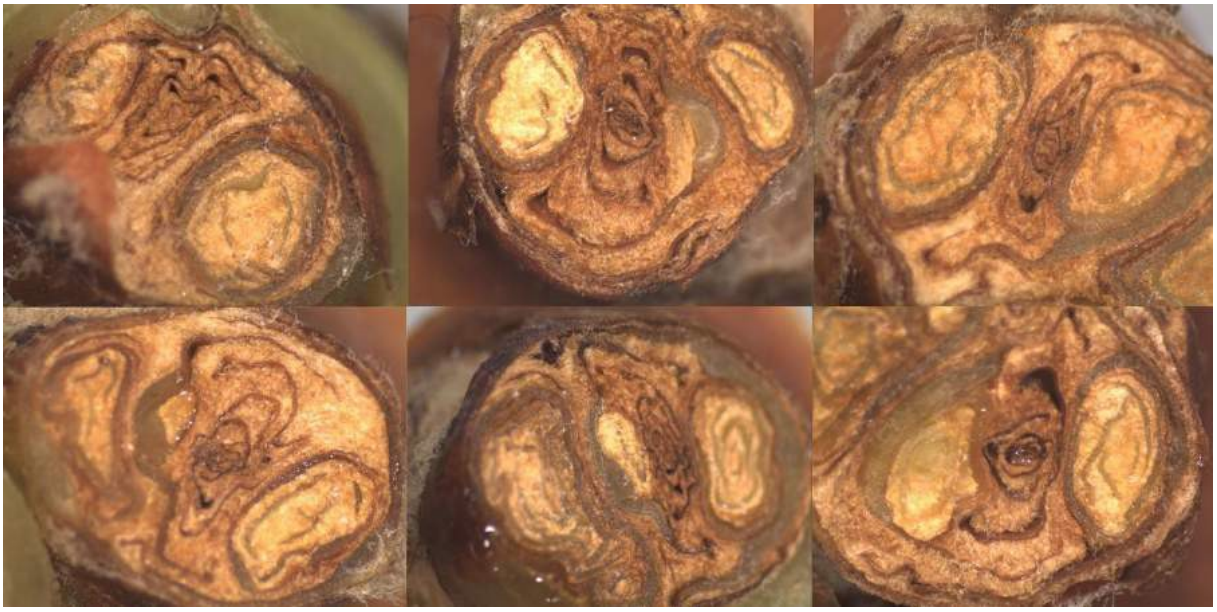


Fig. S6: Microscopic images of compound buds sampled during the experiment, exhibiting primary bud necrosis (PBN).

Appendix 5

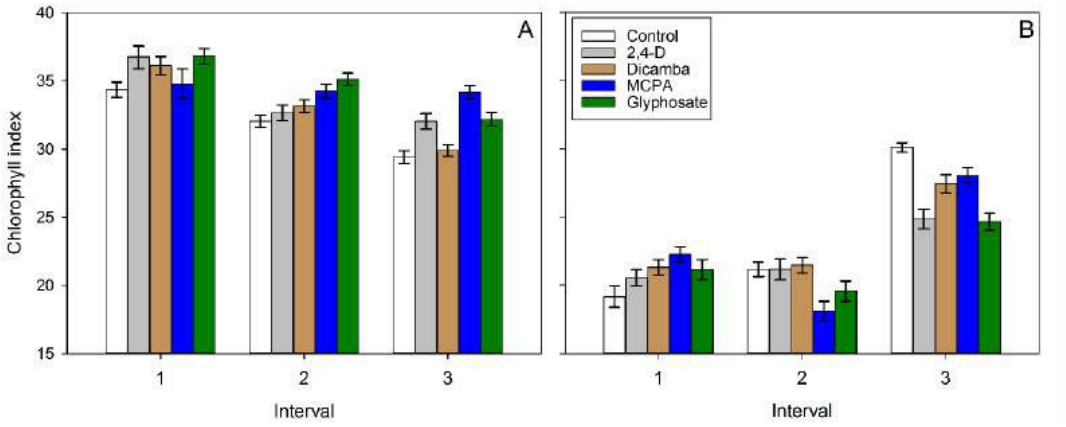


Fig S7. Impact of herbicide exposure at the cessation of flowering on chlorophyll index (CI) of bunch zone leaves (A) and apical leaves (B) during Intervals 1 (post-flowering to peppercorn sized berries), 2 (peppercorn sized berries to véraison) and 3 (véraison to fruit maturity) (mean ± SE).

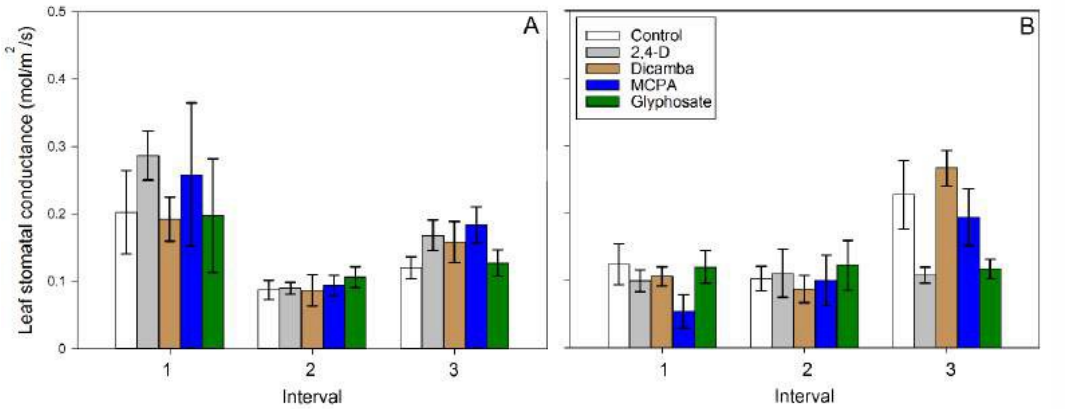


Fig S8. Impact of herbicide exposure at the cessation of flowering on stomatal conductance (g_s) of bunch zone leaves (A) and apical leaves (B) during Intervals 1 (post-flowering to peppercorn sized berries), 2 (peppercorn sized berries to véraison) and 3 (véraison to fruit maturity) (mean ± SE).

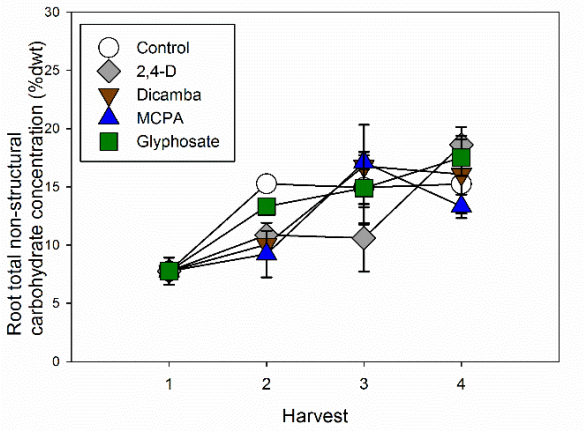


Fig S9. Impact of herbicide exposure on root total non-structural carbohydrate concentration (% dry weight) by each destructive harvest.

Appendix 5

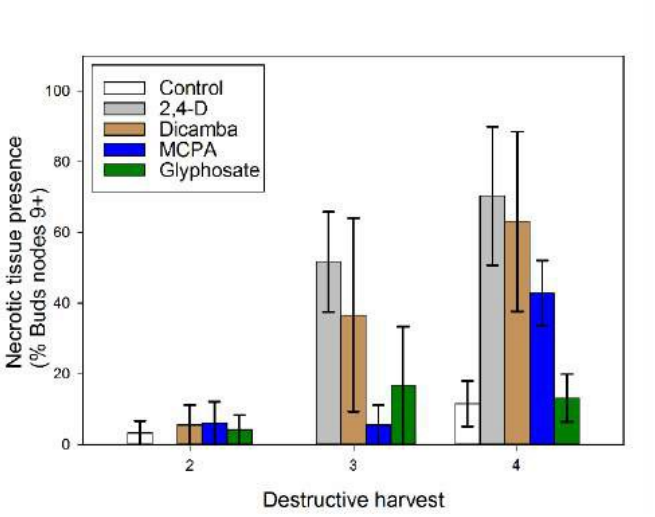


Fig S10. Impact of herbicide exposure on percentage buds on shoot node position nine and above containing necrotic tissues by each destructive harvest.