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A FIELD STUDY ON SOME EFFECTS OF GLYPHOSATE ON *FUSARIUM* SPP.: ITS IMPACT ON ROOT COLONIZATION OF WEEDS, ON PROPAGULE LEVELS IN THE SOIL, AND ON CROP EMERGENCE

by

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B.Sc. Agr. (Great Distinction),

Macdonald College of McGill University, 1983

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A field study on some effects of glyphosate on Fusarium spp.: its impact on root colonization of weeds, on propagule levels in the soil, and on crop emergence.

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June 20, 1985

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ABSTRACT

Glyphosate (Roundup®) is a broad spectrum herbicide that can suppress some defense mechanisms in higher plants. It has been demonstrated that root colonizers can play an important role in the herbicidal action of this herbicide. For these reasons, and because glyphosate can cause root rot-like damage on crops, this study was undertaken to investigate the effect of glyphosate on the soil-borne root colonizing *Fusarium* spp.

The research was conducted at 2 sites. Site # 1 was densely covered with perennial weeds, and site # 2 with annuals. At site # 1, spraying the weed cover with glyphosate increased ($p \leq 0.05$) the level of colonization by *Fusarium* spp. in *Ranunculus repens* L. and *Holcus lanatus* L., but not in *Stellaria media* (L.) Vill. and *Plantago lanceolata* L. At site # 2, glyphosate enhanced colonization in *Spergula arvensis* L., *Stellaria media* (L.) Vill., *Echinochloa crusgalli* (L.) Beauv., and *Chenopodium album* L., but not in *Capsella bursa-pastoris* (L.) Medic. and *Polygonum persicaria* L. At both sites, the number of colony forming units of *Fusarium* spp./g of dried soil was increased by the application of glyphosate. Nevertheless, crops that were sown in the field containing the annual weeds were not detrimentally affected by glyphosate treatment of these weeds.

From these results, and from evidence in the literature, it is concluded that rapid colonization by *Fusarium* spp. of some weed species occurs following treatment with glyphosate and causes an increase in the number of propagules of *Fusarium* spp.

in soil. Current and future uses of glyphosate are discussed in relation to the management of diseases that are a result of weed control practices.

RÉSUMÉ

Le glyphosate (Roundup®) est un herbicide qui peut détruire presque toute végétation. Il a été démontré que les champignons pathogènes du sol jouent un rôle important dans l'action herbicidale du glyphosate. Pour ces raisons et aussi parce que le glyphosate peut endommager les cultures en provoquant une pourriture de leurs racines, cette étude sur les effets du glyphosate sur le genre *Fusarium*, un champignon d'origine édaphique dont font parties plusieurs espèces pathogènes, a été entreprise.

Le travail de champ eut lieu à 2 endroits. Il y avait une forte densité de mauvaises herbes vivaces au site # 1 et d'annuelles au au site # 2. Au site # 1, l'application du glyphosate a augmenté ($p \leq 0.05$) le taux de plantes envahies par *Fusarium* spp. chez *Ranunculus repens* L. et *Holcus lanatus* L., mais cet effet n'a pas été observé chez *Stellaria media* (L.) Vill. et *Plantago lanceolata* L. Au site # 2, le glyphosate a eu l'effet d'accroître le taux de plantes envahies par *Fusarium* spp. chez *Spergula arvensis* L., *Stellaria media* (L.) Vill., *Echinochloa crusgalli* (L.) Beauv., et *Chenopodium album* L., mais tel ne fut pas le cas pour *Capsella bursa-pastoris* (L.) Medic. et *Polygonum persicaria* L. Aux 2 sites, l'application du glyphosate sur les mauvaises herbes a accru le nombre de germes des *Fusaria*/g de sol sec. Néanmoins, l'application du glyphosate sur les mauvaises herbes n'a pas affecté négativement l'émergence des cultures semées au site # 2.

A partir de ces résultats ainsi que d'autres publiés par divers auteurs, il est conclu que les tissus des plantes traitées au glyphosate sont rapidement envahis par *Fusarium* spp. et qu'un accroissement du nombre de germes de *Fusarium* spp. dans le sol s'ensuit. Les perspectives d'avenir du glyphosate sont discutées en relation avec la protection des cultures contre les maladies issues des techniques de contrôle des mauvaises herbes.

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I want to express my profound gratitude to my brother Louis for his skilful assistance during the last summer field and laboratory work and to Line, my wife, for her everyday support and helping hand.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

Glyphosate (Roundup®) is a water soluble herbicide that kills most herbaceous plants when applied as a foliar spray (Monsanto, 1982). In many annual and perennial species, this herbicide is rapidly absorbed and translocated downward through the vascular tissues into the roots (Sprankle *et al.*, 1975c; Coupland *et al.*, 1979 & 1981). About 3 days after treatment, respiration and photosynthesis are gradually inhibited and approximately one week later chlorosis is apparent (Sprankle *et al.*, 1975c). Glyphosate has a rapid effect on many biochemical processes taking place in higher plants (Hoagland and Duke, 1982; Cole *et al.*, 1983), but the only effect proven to be a primary mode of action is the inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase involved in the shikimic acid pathway (Steinrücken and Amrhein, 1980 & 1984; Rubin *et al.*, 1984). This pathway leads to the synthesis of phenylalanine, from which phytoalexins are derived (VanEtten and Pueppke, 1976; Dewick and Steele, 1982). Phytoalexins are involved in mechanisms of disease resistance of plants (Deverall, 1977; Darvill and Albersheim, 1984).

These facts comprise the rationale for a study in which glyphosate is being used to investigate the association of phytoalexin production with resistance of bean plants, *Phaseolus vulgaris* L., to anthracnose, *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scribner. During the early stages of this

investigation, Johal and Rahe (1984) showed that bean plants treated with a certain dose of glyphosate died when grown in non-sterile soil, but survived in sterilized soil. They demonstrated clearly that the death of the treated bean plants was due to fungal colonization of roots, principally by *Pythium* spp. and *Fusarium* spp. Lynch and Penn (1980), as well as Brown and Sharma (1984) showed that glyphosate-treated plants, respectively, quackgrass, *Agropyron repens* (L.) Beauv., and flax, *Linum usitatissimum* L., were rapidly colonized by fungi. However, they did not link the fungal colonization with the herbicidal action.

Many herbicides influence negatively or positively the incidence of diseases on crops (Katan and Eshel, 1973; Altman and Campbell, 1977; Griffiths, 1981; Smith, 1982). The interactions that have been investigated the most are:

1. Herbicide-pathogen,
   a. effect on growth,
   b. effect on the virulence of the pathogen.
2. Herbicide-host,
   a. effect on defense mechanisms,
   b. effect on exudation from host tissues.
3. Herbicide-antagonists or competitors-pathogens, the indirect effect on pathogens through inhibition or enhancement of competitors or antagonists.

Herbicides also indirectly affect crop diseases by eliminating weeds and cause changes in microclimate that modify

the incidence of diseases on crops (Kavanagh, 1969 & 1974). They can make crop rotation more effective by removing secondary hosts of pararitic nematodes when the susceptible crop is absent (Franklin, 1970). Herbicides can also modify the incidence of some plant virus diseases by eliminating a source of inoculum (Heathcote, 1970).

Lynch and Penn (1980) demonstrated that glyphosate had an indirect effect on crops via the populations of root colonizers that build up on the treated weeds. From their laboratory experiment, they concluded that *Fusarium culmorum* (W.G.Sm.) Sacc., which had colonized quackgrass rhizomes after the application of glyphosate, as well as some toxins liberated by the subsequent decay, were the agents that damaged barley, *Hordeum vulgare* L. var Proctor. Oswald (1980) demonstrated that glyphosate applied at different concentrations did not seem to be transferred from the roots of the treated broad-leaved dock, *Rumex obtusifolius* L., to the roots of some selected plant species. Coupland and Lutman (1982) showed that glyphosate could be transferred from the rhizomes of quackgrass to adjacent wheat roots, *Triticum aestivum* L., at high dose levels, but this effect was not observed when glyphosate was sprayed at field dose levels. Therefore, it is unlikely that the damage observed on crops after the application of glyphosate for weed control was due to the uptake of residual herbicide by the crops. Still, very little is known about the indirect effects of glyphosate on crops.

My objective was to investigate in the field some aspects of the indirect effects of glyphosate on *Fusarium* spp. and on crops. *Fusarium* spp. were the most common fungal colonizers at the 2 sites studied: in sites 1 and 2, 58 of 158 and 134 of 416 fungal isolates, respectively, were identified as *Fusarium* spp. Glyphosate was the ideal candidate from among the large number of various herbicides by reasons of its mode of action that suppresses some defense mechanisms in a broad range of plants (Gresshoff, 1979) and the possibility that its herbicidal action is partially mediated by root colonizers (Johal and Rahe, 1984). The hypotheses to be investigated were:

1. that *Fusarium* spp. colonize some or all the weed species treated when glyphosate is applied at the recommended levels, and
2. that the associated increase in fungal propagules in the soil is dependent on the time elapsed between spraying and time of observation, or between spraying and tillage.
3. *Fusarium* spp. enhanced by glyphosate treatment of weeds can damage crops.

Materials and Methods

Site = 1

This site is an abandoned field near Aldergrove, British Columbia. The field had a dense cover of predominantly perennial grasses and broadleafed weeds, and an annual weed (*Stellaria media* (L.) Vill.) that successfully overwintered (Table I). The soil was a Columbia loamy sand. The treatments (Table II) were applied on 2x6 m plots. They were replicated 5 times and laid out in a randomized complete block design. On June 12, 1984, glyphosate was applied with a back pack Solo® sprayer at the rate recommended for the control of perennial weeds, *i.e.* 7.0 L of Roundup®/ha (2.52 kg a.i./ha).

Three, 11 and 18 d after spraying, one plant of each of 4 weed species (Table I) was randomly sampled from every non-tilled plot. For each plant, the following procedure was executed: the roots were washed in running water for approximately 2 min Three 1 cm pieces of root were excised from the upper portion of the root zone (sections of young lateral or adventitious roots taken 2-8 cm below soil line), and, except for *S. media* from which only root pieces were taken, three 1 cm pieces were cut from the base of the stem. These pieces were surface sterilized for 2 min in 1% NaOCl, plated on potato dextrose agar (PDA), and incubated in dark at 23±2°C. After 4 d

Table I List[1] of the weed species sampled in sites 1 and 2.

Site	Common name	Latin name	Annual(A) Perennial(P)
1	Velvet grass	*Holcus lanatus* L.	P
	Chickweed	*Stellaria media* (L.) Vill.	A
	Narrow-leaved plantain	*Plantago lanceolata* L.	P
	Creeping buttercup	*Ranunculus repens* L.	P
2	Shepherd's-purse	*Capsella bursa-pastoris* (L.) Medic.	A
	Chickweed	*Stellaria media* (L.) Vill.	A
	Corn spurry	*Spergula arvensis* L.	A
	Lady's-thumb	*Polygonum persicaria* L.	A
	Barnyard grass	*Echinochloa crusgalli* (L.) Beauv.	A
	Lamb's-quarters	*Chenopodium album* L.	A

[1] The names appear in decreasing order according to density.

Table II List of treatments applied in sites 1 and 2.

Site	Treatment number	Application of glyphosate	Tillage
1	1	None	None
	2	Yes	None
	3	Yes	1 week after spraying
	4	Yes	2 weeks after spraying
	5	Yes	3 weeks after spraying
	*	*	*
2	1	None	16 days before seeding[1]
	2	16 days before seeding[1]	None
	3	9 days before seeding	None
	4	At seeding	None
	5	Just before emergence	None

[1] All plots seeded between July 8 and July 12.

the tissue pieces were scored for presence or absence of *Fusarium* spp. For any ambiguous cases (absence of spores, contamination, etc.), hyphal tips of fungal colonies were transferred to a *Fusarium*-selective medium (Nash and Snyder, 1962). Single spore cultures representing the major morphotypes of *Fusarium* spp. isolated were sent to the Biosystematics Research Institute, Agriculture Canada, Ottawa for identification to species. The logistic regression (Lee, 1980; Engelman, 1983) was used for analysis of data on colonization of weed species by *Fusarium* spp.

Three and 9 w after spraying, a composite soil sample made up of 15, 2.5x10 cm cores was collected from every plot. The samples were kept at about 4°C until processing. In the laboratory, each composite sample was mixed again and 2 subsamples were taken with a sterile spatula which excluded organic debris or stones larger than approximately 3-4 mm diameter. The first subsample was used to determine soil moisture level and the second for dilution plating. For determination of soil moisture, a 5-10 g subsample was weighed at ±0.0001 g before and after 6 h of drying at 105°C. For dilution plating, a 3-5 g subsample of soil was taken, weighed at ±0.0001 g, transferred into 100 ml of 0.1% agar suspension in distilled water, and mixed on a rotary shaking machine for 1 h. One ml was taken from the suspension and transferred to 9 ml of 0.1% agar to make a ten-fold dilution. Three higher dilutions were prepared by transferring 1 ml of the last dilution to

another 9 ml of 0.1% agar. Aliquots of 0.5 ml were taken from each dilution for plating on *Fusarium*-selective medium. The plates were incubated at room temperature (23±3°C) and nominally 16 h photoperiod. After verification of their identity, colonies were counted from those dilution plates that contained approximately 20-50 of them. The number of colonies/plate was used to give estimates of colony forming units/g (CFU/g) of dry soil. Data from the non-tilled plots, treatments 1 and 2 (Table II), were analyzed using a paired-sample t-test. The data from the sprayed plots (treatments 2,3,4,5), in randomized complete blocks, were analyzed using an analysis of variance.

Site # 2

The site is a cultivated field at an Agriculture Canada (Agassiz) substation near Abbotsford, British Columbia. The field had a dense cover of annual grasses and broadleafed weeds as of June 1984. The soil was a silt loam. Five treatments (Table I) were applied on 6x10 m plots that had been laid out in a Latin square design. Glyphosate was applied beginning on June 25, 1984, at the rate recommended for annual weeds, *i.e.* 3 L of Roundup®/ha (1.08 kg a.i./ha).

Three plants from each of 6 weed species (Table I) were randomly sampled from each glyphosate-treated plot of row 3 of the field design. The sampling was done at 0, 4 , 7 and 14 d after application of the herbicide (Fig. 1). The plants were processed like the ones sampled in site # 1, and the data on

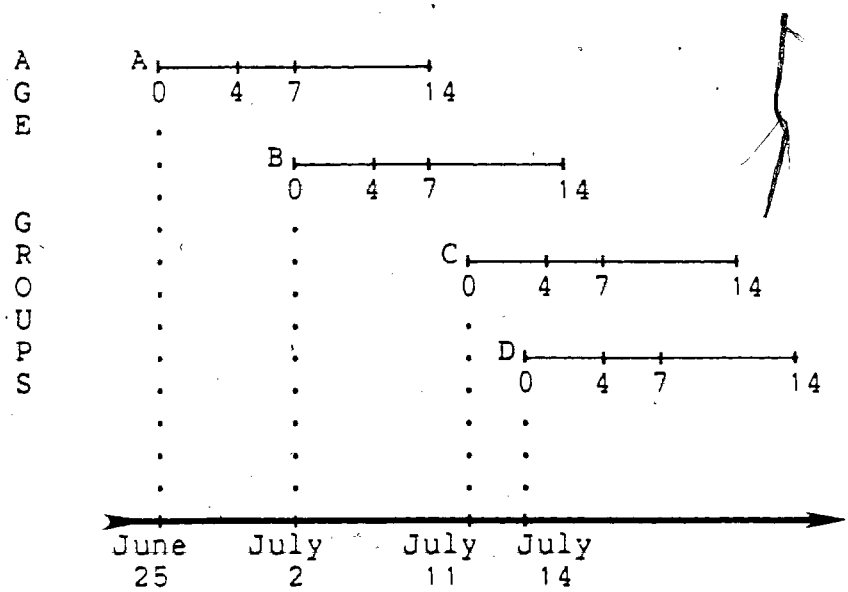

Fig. 1 Spraying and sampling schedule in site # 2. Each block or age group was sprayed on one of the 4 dates mentioned. In each block, plants were sampled just before, and 4, 7, and 14 d after spraying.

colonization of weed species by *Fusarium spp.* analyzed using the logistic regression.

In every plot, 100 seeds of each of 4 crops (Table III) were sown; all plots were seeded between July 8 and July 12. Each seed was individually planted at a depth of 4 cm, and spaced by 2.5 cm within and 1 m between the rows. Each 6x10 m plot was divided lengthwise into 4 subplots so that 4 rows of 2.5 m could be allocated to each crop. The emerging plants were counted 2 w after seeding. and data about the proportion of planted seeds that emergenced were subjected to arcsine transformation and analysis of variance for Latin square designs. Significant F-values ($p \leq 0.05$) resulting from the analysis of variance were processed using the Newman-Keuls test.

On June 22 (before any treatement was applied), July 18 (just before crops emergence), August 8, and September 7, soil samples were taken from every plot for assessment of propagule levels of *Fusarium* spp. The sampling and processing techniques were as described for site #1. The data were subjected to a multiple non-linear regression analysis (Gomez and Gomez, 1984; Frane, 1983).

Table III List of crops planted[1] in site # 2.

Common name	Latin name	Variety
Bush bean	*Phaseolus vulgaris* L.	Topcrop
Sweet corn	*Zea mays* L.	Sunnyvee
Cucumber	*Cucumis sativus* L.	Marketer
Common pea	*Pisum sativum* L.	Littel Marvel

[1] Planting done between July 8 and July 12.

Results

Effect of glyphosate on colonization of weeds by *Fusarium* spp.

a) Abandoned field, Site # 1

The rate of colonization of *Ranunculus repens* and *Holcus lanatus* by *Fusarium* spp. was significantly higher in glyphosate-treated plants than in the control plants (Fig. 2). However, I did not detect any significant differences in the colonization of *Stellaria media* and *Plantago lanceolata*. *Fusarium avenaceum* Schlecht and *Fusarium oxysporum* (Fr.) Sacc. in a ratio of 5:1 comprised over 95% of the total *Fusarium* spp. recovered.

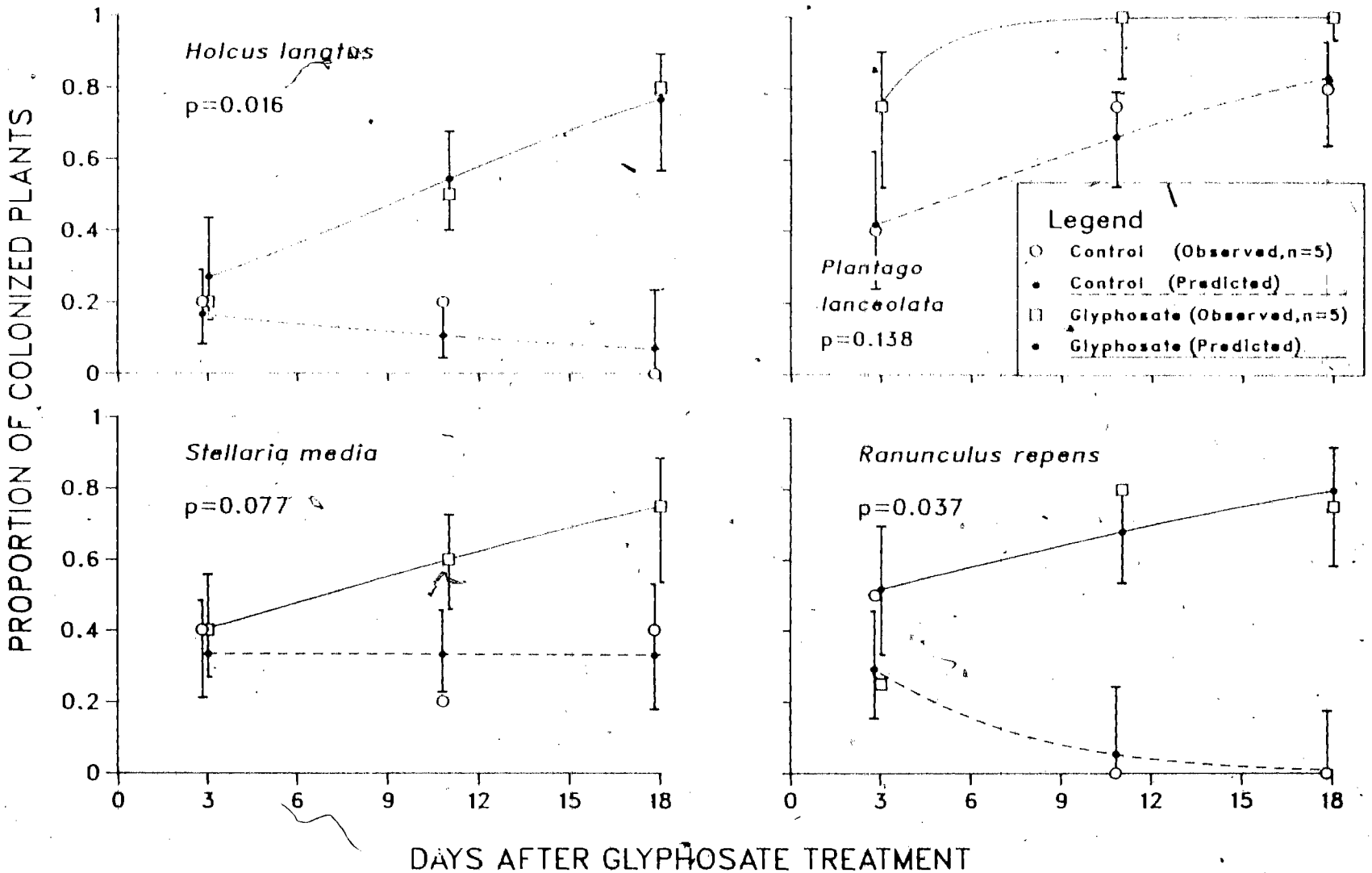

Fig.2 Proportion of plants from site # 1 colonized by *Fusarium* spp. as a function of time after spraying for selected weed species. The analysis was done on the log odds scale where the relationship between days after treatment and log odds of plant colonization [$\log(\pi/1-\pi)$ where π=proportion of colonized plants] follows a general linear regression model. The estimated theoretical mean (Predicted) ± S.E. in the log odds scale gave an upper and lower limit which were transformed back in the proportion scale in order to give an upper and lower limit about the estimated theoretical mean in the proportion scale (‡). The p-value is the significance level for Ho: (control slope) ≥ (glyphosate slope) as given by t-test.

b)Cultivated field, Site # 2

The p-values of the improvement Chi-Square (Table IV) show the significance of adding new predictor variables (independent) in the model used for the analysis of the data for each weed species. These p-values fulfil the same purpose as do the ones obtained from the F-value of the significance test for additional independent variables in multiple regression analysis or analysis of variance. For *Capsella bursa-pastoris* and *Polygonum persicaria* the analysis indicated that a single constant was precise enough to represent the data. For *Echinochloa crusgalli*, one regression line with the 4 age groups pooled together was sufficient. As neither the proportions of plants colonized by *Fusarium* spp. just before spraying (intercepts of curves), nor the rates of colonization by *Fusarium* spp. between the age groups (slopes of curves) were significantly different, I conclude that, for *E. crusgalli*, *P. persicaria*, and *C. bursa-pastoris*, and for the period when sampling took place, senescence had no conclusive effect on the proportion of plants colonized by *Fusarium* spp. This was especially so because each age group (or block) was sprayed at different times during a portion of the growing season (Fig. 1).

For *Spergula arvensis*, *S. media*, and *Chenopodium album*, the analysis demonstrated that the best model was one allowing different time-rates of change in the proportion of plants colonized by *Fusarium* spp. (4 different slopes), and different

Table IV P-values of the improvement Chi-squares testing the hypothesis that the newly added set of predictor variables significantly improved the prediction of the dependent variable (effect of glyphosate on the proportion of plants of one weed species colonized by *Fusarium* spp.) from the previous model.

Weed species	P-values		
	Model 2[1]: 1 slope & 1 intercept (1 d.f.)	Model 3: 4 slopes & 1 intercept (3 d.f.)	Model 4: 4 slopes & 4 intercepts (3 d.f.)
Capsella bursa-pastoris	0.296	0.214	0.579
Stellaria media	0.002	0.066	0.002
Spergula arvensis	0.004	0.544	0.008
Polygonum persicaria	0.551	0.145	0.275
Echinochloa crusgalli	0.013	0.398	0.423
Chenopodium album	0.034	0.011	0.056

[1] Model 1: 1 intercept (one curve with slope=0)

proportions of colonized plants just before spraying (4 different intercepts) for the 4 age groups. However, because the proportion of colonized plants observed before spraying did not increase consistently with time (Table V), I conclude that these data did not reveal any significant increase in colonization in *S. media*, *S. arvensis*, and *C. album* during the time period when the study ocurred. Consequently, for every species studied, the data from each block were pooled to illustrate the effect of glyphosate on root colonization by *Fusarium* spp. (Fig. 3).

The effect of glyphosate on the rate of colonization was significant (slope > 0) in *S. media* , *S. arvensis*, *E. crusgalli*, and *C. album*, while no significant increase was detected in *C. bursa-pastoris* and *P. persicaria*.

In site = 2, *F. oxysporum* and *F. avenaceum* in a ratio of 1:1 contributed to over 95% of the total *Fusarium* spp. recovered.

Table V Estimated theoritical mean ± S.E. and observed () proportions of weed plants colonized by *Fusarium* spp. before spraying.

Weed species	Estimated theoritical means ± S.E. and observed proportions () by age group (A-D) for the sampling dates at which spraying took place[1]			
	A June 25	B July 2	C July 12	D July 15
Spergula arvensis	0.0±0.0 (0.0)	0.48±0.24 (0.33)	0.0±0.0 (0.0)	0.31±0.21 (0.0)
Stellaria media	0.09±0.11 (0.0)	0.46±0.23 (0.0)	0.0±0.0 (0.0)	0.38±0.22 (0.0)
Chenopodium album	0.86±0.17 (1.0)	0.68±0.21 (0.67)	0.29±0.25 (0.33)	0.0±0.01 (0.0)

[1] Sampling was done just before spraying.

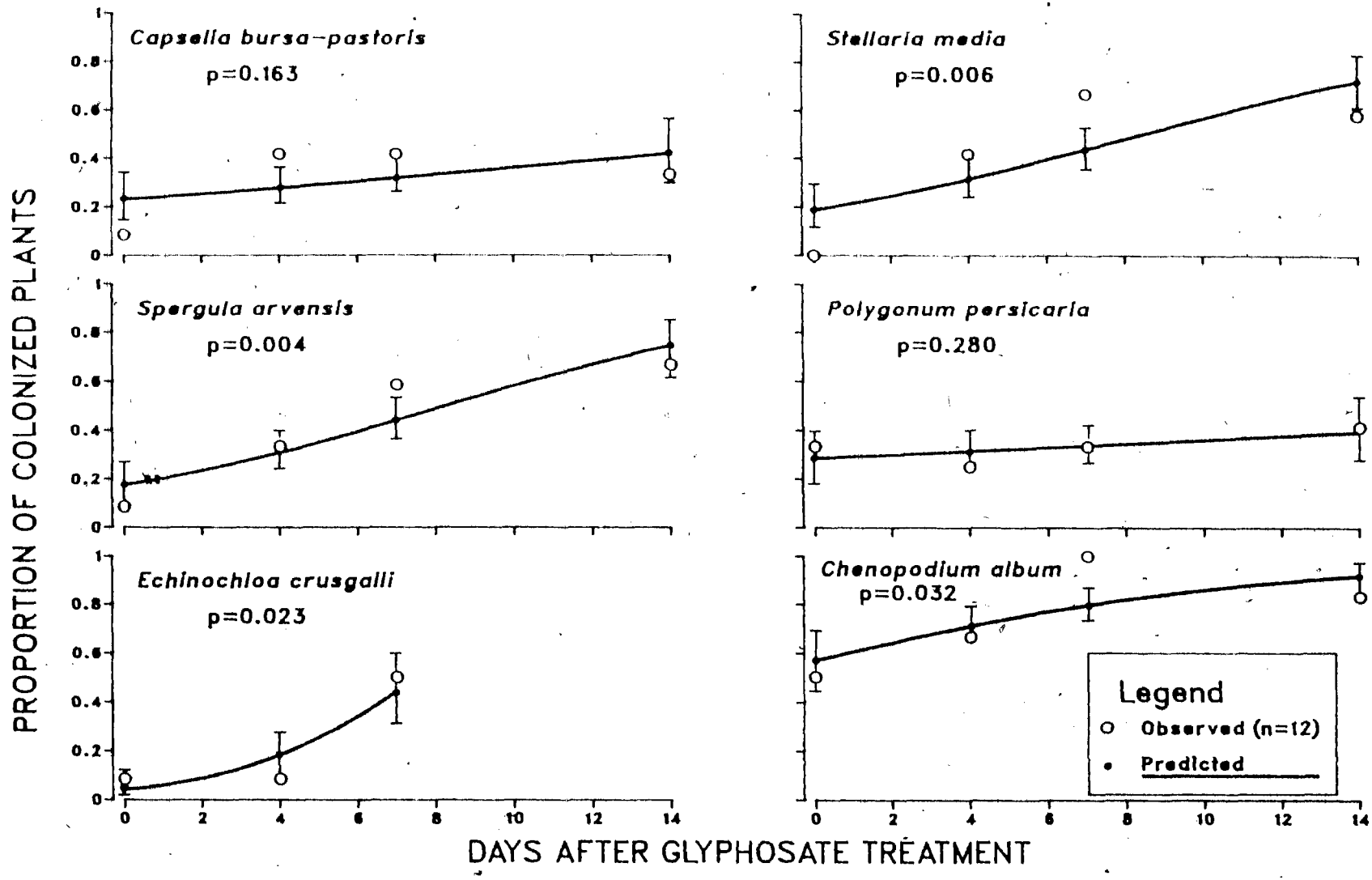

Fig.3. Proportion of plants from site # 2 colonized by *Fusarium* spp. as a function of time after spraying for selected weed species. The analysis was done on the log odds scale where the relationship between days after treatment and log odds of plant colonization [$\log(\pi/1-\pi)$ where π=proportion of colonized plants] follows a general linear regression model. The estimated theoretical mean (Predicted) ± S.E. in the log odds scale gave an upper and lower limit which were transformed back in the proportion scale in order to give an upper and lower limit about the estimated theoretical mean in the proportion scale (‡). The p-value is the significance level for Ho: slope ≤ 0 as given by t-test.

Propagules of *Fusarium* spp. in soil

a)Abandoned field, Site # 1

Three weeks after spraying, the number of CFU/g of *Fusarium* spp. was significantly higher in the sprayed plots compared with the control plots (Fig. 4). Nine weeks after spraying, that difference was less but still significant.

Tillage of the sprayed plots at different intervals after spraying did not have any significant effect on levels (CFU/g) of *Fusarium* spp. (Fig. 5) in the soil.

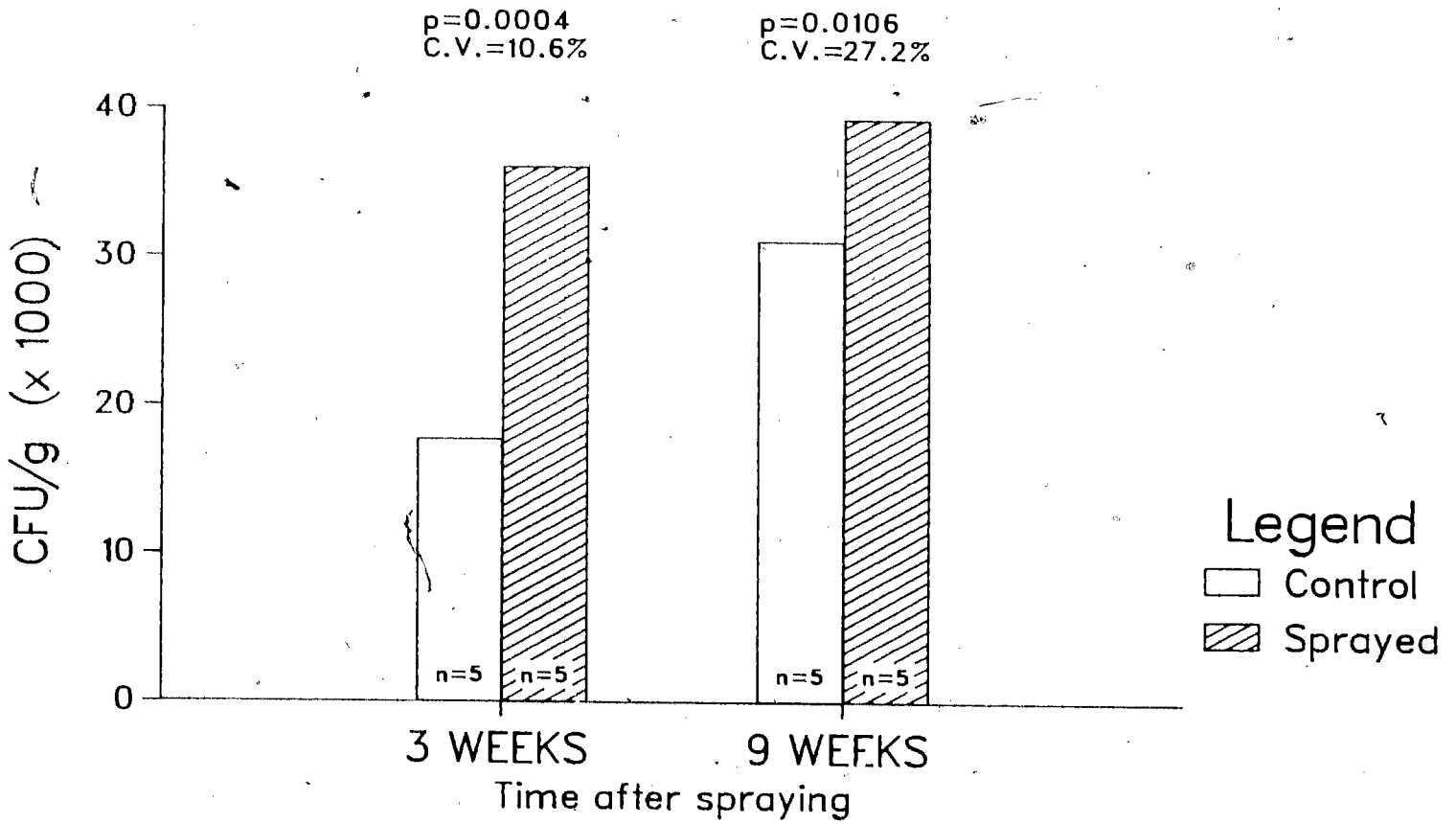

Fig.4 Levels of *Fusarium* spp. in sprayed and control plots, as observed at two different times after the application of glyphosate. The p-value is the significance level for Ho: [(CFU/g in control)-(CFU/g in sprayed plots)] $\leq$ 0 within each time after spraying as given by a paired-sample t-test. C.V. is the coefficient of variability.

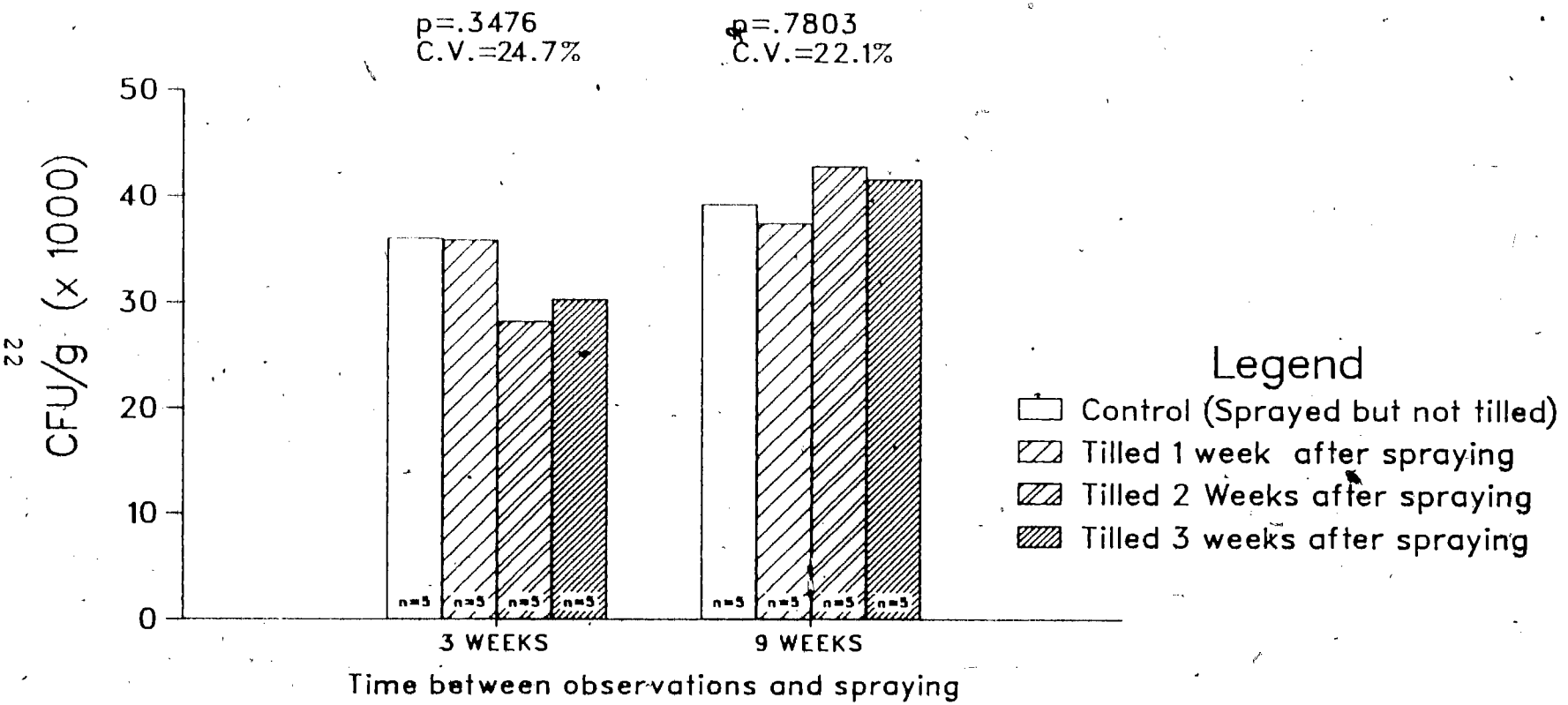

Fig.5 Effect of time elapsed between tillage and spraying on level of *Fusarium* spp. The p-value is the significance level for Ho: all means are equal within each time between observations and spraying. C.V. is the coefficient of variability.

b)Cultivated field, Site # 2

During the season, the level (CFU/g) of *Fusarium* spp. in the control plots fluctuated significantly. In Fig. 6 one can visualize what was observed in the control plots by following the curve along the axis of time of observation when spraying time equals zero. For each time of observation, one can view the effect of spraying by following the curves that are parallel to the axis of the time after spraying. Glyphosate treatment significantly increased the level of *Fusarium* spp. present in the soil. A maximum was reached 3 weeks after spraying. There followed a decline, and another increase near the end of the growing season (end of August).

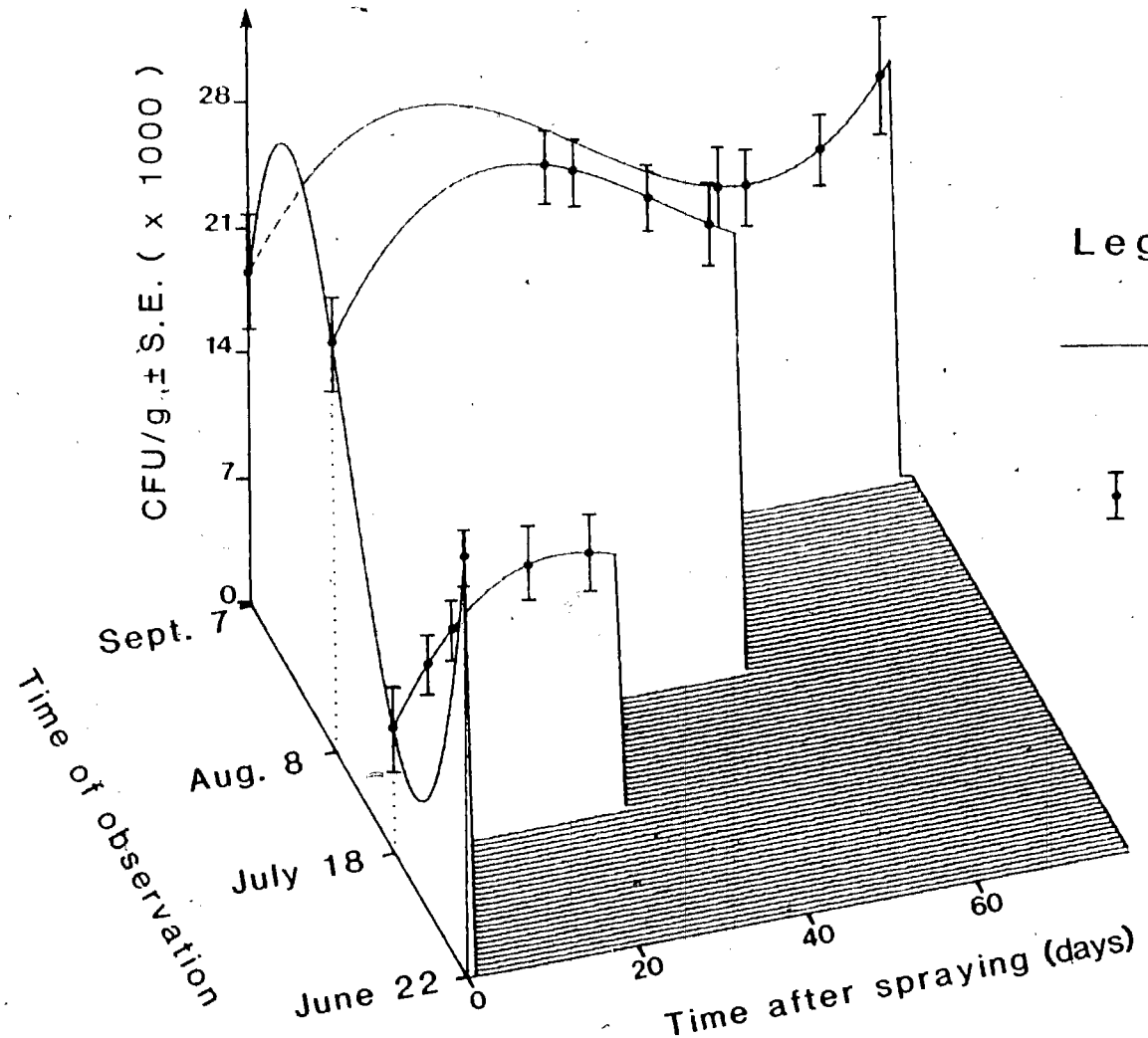

Fig.6 At site # 2, level of *Fusarium* spp. in soil (CFU/g) as a function of time of observation during the growing season, as well as time after spraying glyphosate.

Crop emergence

The emergence of *Cucumis sativus* L. and *Phaseolus vulgaris* L. was not affected significantly by any of the treatments (Fig. 7). For *Pisum sativum* L., the emergence was the highest in the plots sprayed 9 d before seeding. For *Zea mays* L., a significantly higher emergence occurred in all sprayed plots than in the control plots.

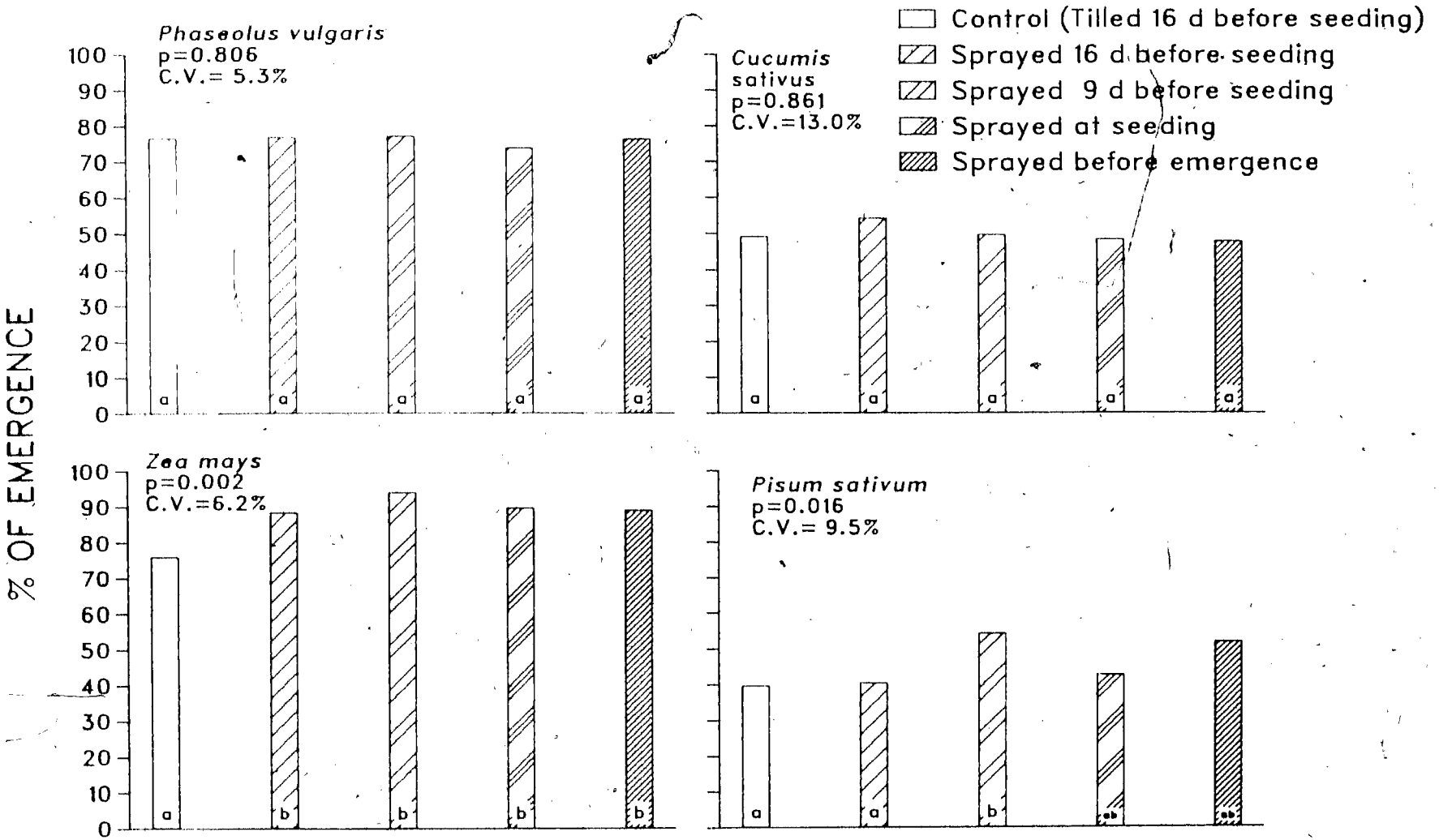

Fig. 7 Percentage germination evaluated 2 w after seeding, as affected by interval between spraying and seeding. For beans, a count made 3 w later is reported since some plants were not emerged 2 w after seeding. Within each crop, the percentages represented by the same letter(s) are not significantly different at the .05 level. C.V. is the coefficient of variability. For each bar, n=500.

Discussion

Colonization of weeds

During the portion of the season under study, *Fusarium* spp. were recovered from tissues of certain plants that had not been treated with glyphosate. Recoveries were particularly frequent in the plants of the abandoned field (site # 1), as well as in *P. persicaria* and *C. album* growing in the cultivated field (site # 2). In *P. lanceolata*, natural colonization of control plants by *Fusarium* spp. increased during the period of observation to a level as high as that observed in the glyphosate-treated plants. Nevertheless, the plants that were not treated with glyphosate, but colonized by *Fusarium* spp. looked vigorous and healthy.

Fusarium spp. are omnipresent in agricultural soils (Gordon, 1956 & 1960), and they attack a wide range of hosts (Booth, 1971). It is not surprising to find *Fusarium* as a colonizer of plants, particularly weeds, that have overwintered. Kreutzer (1972) frequently isolated *Fusarium* spp. from the rhizosphere, the rhizoplane, and the inner root of many grassland species. *Fusarium* spp. will have a better chance to dominate the niche created by plant senescence if they establish as weak parasites when the plants are still vigorous and healthy. Cook and Bruehl (1968) demonstrated that wheat straw not invaded parasitically by *Fusarium culmorum* remained

unoccupied by the fungus after plowing, whereas the plants that had been colonized through parasitism still harbored the fungus many weeks after plowing.

As the proportion of weed plants colonized by *Fusarium* spp. never decreased with the application of glyphosate, it can be concluded that glyphosate did not reduce the competitive advantage of the *Fusarium* colonizers that were established within host tissues prior to glyphosate treatment.

Except for *P. persicaria*, all the plants treated with glyphosate died and were eventually colonized by some fungi and/or bacteria. In some weed species, glyphosate treatment significantly increased the proportion of plants colonized by *Fusarium* spp., even though *Fusarium* was never able to colonize all the plants of a given species (Figs. 2, 3). This inability could result from one or a combination of factors relating to genetic variability, chance variations, and non-quantitative aspects of the recovery technique. De Gennero (1984) established that different biotypes of *Convolvulus arvensis* L. responded differently to glyphosate treatment. Variations, in dose received, phenologic stages of the plants at time of spraying, as well as variations in microflora and microenvironment surrounding the plant, could all contribute to the observed differences between and within species.

I believe that recoveries are an accurate estimate of the presence of *Fusarium* spp. in tissues. The *Fusarium* spp. isolated have the ability to grow extensively within vascular tissues: at

the last sampling, for each plant in which *Fusarium* spp. was observed, the fungus was consistently isolated from most of the plant pieces plated. An important factor that can interfere with detection of *Fusarium* spp. from infected plants is oversterilization: colonizers increase the permeability of tissues, and thus, excessive surface sterilization can result in complete sterilization. This effect would most likely become a factor in species that have tiny roots or stems such as *S. media* or *S. arvensis*. However, at site # 2, *S. media* and *S. arvensis* were the weed species that showed the most significant increase in colonization by *Fusarium* spp. after glyphosate treatment. Therefore, I conclude that oversterilization did not affect significantly the recoveries of *Fusarium* spp. from infected tissues.

The major possible source of bias in this investigation came from the random sampling technique for the weed plants. At the time of the last sampling in both sites, many of the sprayed weed plants were simply gone. Consequently, the probability of sampling weed plants that were less affected by glyphosate treatment was increased proportionally to time after spraying. This explains why *E. crusgalli* could not be sampled at day 14, and also the drop in observed proportion of colonized plants at the last sampling in several weed species.

Fusarium spp. are well adapted to establish themselves parasitically within weakened plants (Cook, 1969). Glyphosate treatment provided an advantage to *Fusarium* spp. by facilitating

the colonization of some of the weed species studied. This is further supported by the fact that the *Fusarium* spp. isolated are known plant pathogens and also by the work of Johal and Rahe (1984) concerning the role of plant pathogenic fungi on the herbicidal action of glyphosate: metalaxyl, a fungicide specific against pythiaceous fungi and inactive against *Fusarium* spp. blocked the herbicidal effect of glyphosate in the presence of *Pythium* spp. but not *Fusarium* spp.

Level of *Fusarium* in the soil

Wainbridge (1982) showed clearly that, in the dilution technique, the colony counts were largely representing the fungal spores. Therefore, in the following discussion, unless otherwise stated, *Fusarium* level, propagules, or CFU/g, refer to the number of viable conidia or chlamydospores/g of dried soil as manifested by colony production..

At both sites, the *Fusarium* spp. level in the control plots fluctuated during the season (Fig.6). In the field with annual weeds (site #2), the extremely dry conditions that began about the beginning of June could explain the decrease in propagules observed during the period from June 22 to July 18 (Fig.6). The subsequent increase could be partly explained by the remoistening of the dried soil, a process that is known to release nitrogenous materials, and consequently cause a sudden increase in number and activity of microorganisms (Stevenson, 1986). In addition, decay of the tilled material in the control

plots of site #2, and of the naturally senescent plants in site #1, may have contributed to the observed increases? Naturally occurring increases in soil microbial activity have been observed in the middle of the summer by Snyder and Nash (1968).

At both sites, the level of *Fusarium* spp. was significantly increased in the sprayed plots when compared with the control plots. Maximum differences occurred at 3 weeks after spraying at both sites, but this time of maximum difference is better supported for site # 2 because many samples were taken at regular intervals for 74 days after spraying.

In site #2, the observed increase in *Fusarium* level was followed by a decrease and then a second increase. I can only speculate about the reasons for this phenomenon. Increased activity of some microorganisms antagonistic to *Fusarium* spp. may have occurred as a delayed response to some effects of spraying with glyphosate. Antagonistic activity could have contributed to the observed decrease in *Fusarium* levels. The second increase might have resulted from a decline in antagonism as whatever factor responsible for its increase was exhausted. The observed increase and decrease of *Fusarium* spp. levels due to antagonistic microorganisms could have been synergistically affected by fluctuations in percentage germination of conidia or chlamydospores of *Fusarium* spp. It has been demonstrated that as conidial or chlamydospore density of *Fusarium solani* (Mart.) Sacc. increases, the percentage germination of those propagules decreases (Griffin, 1970; Griffin and Ford, 1974). The second

increase could as well have been provoked by the colonization of the weeds that emerged after the application of glyphosate: some of these weeds were senescent when the second increase was observed.

In site #1, it was expected that tillage would favor the production and the distribution of *Fusarium* propagules. This outcome was not observed, possibly because the roots formed such a compact mass at this site, and propagules of *Fusarium* were already well dispersed within the undisturbed sod.

The application of a herbicide over a certain area creates so many changes in the environment that it becomes difficult to pinpoint the cause of an observed fluctuation in the microflora (Smith, 1982). Nevertheless, for the following reasons, I conclude that the number of propagules of *Fusarium* spp. increased in the soil after they had built up on the tissues of some of the weeds that had been treated with glyphosate:

1. *In vitro*, glyphosate inhibits the growth of many pathogens (Harris and Grossbard, 1979; Stedman, 1982) including *Fusarium* spp. (Brown and Sharma, 1984).
2. When incorporated directly into different types of soil, glyphosate has no significant effect on either total number of microorganisms, emission of CO_2, or N uptake of those soils (Cérol et Seguin, 1982).
3. Since the mobility of glyphosate is very limited in the soil (Sprankle *et al.* 1975 a,b), and a very small fraction of what is applied is exuded from the roots of treated weeds

(Coupland *et al.*, 1979 & 1981), it is unlikely that glyphosate, when applied at a normal field dose, would be more inhibitory to antagonists or competitors of *Fusarium* spp. than to *Fusarium* spp. *per se*.

4. The increase in *Fusarium* spp. level was observed in 2 sites where conditions were different:
   a. In site #1 where the control and the sprayed plots were left undisturbed, the first significant difference was observed when the canopies made by the sprayed plants were chlorotic but still dense. Canopy removal is one of the major causes of environmental changes induced by herbicides. This environmental modification had not occurred when the significant increase was observed.
   b. In site #2, the control plots were tilled and the sprayed plots left undisturbed. If anything, the tillage should have increased the *Fusarium* levels in the control plots by enabling the fungus to build up on the plants that were colonized before tillage. Moreover, *Fusarium* spp. are not adapted to anaerobic conditions (Stover, 1953), therefore tillage would not have been detrimental in that respect.
5. The application of glyphosate was followed by increases in the proportion of plants colonized by *Fusarium* spp. in the majority of the weed species studied. As those plants died, *Fusarium* spp. likely exploited their advantage as pioneer colonists in order to build up their inoculum levels.

Crop emergence

I demonstrated that in a field with a dense cover of annual weeds, regardless of the length of the interval between spraying and seeding, crop emergence in glyphosate-treated plots was no lower than in tilled plots. On the emerged crop seedlings, there were no visual signs or symptoms of parasitism by *Fusarium* spp. It is possible that an effect on yields could have been observed, but the weed competition varied so much between treatments that the results would have been meaningless. In order to obtain conclusive results about growth parameters, the newly emerging weeds should have been removed by hand as they were coming out. Unfortunately, the human resources involved in this research project were insufficient to accomplish this task.

The lack of a decrease of crop emergence due to glyphosate should not be extrapolated to other conditions. Lynch and Penn (1980) reported that glyphosate application to weeds resulted in poor establishment and death of cereals, and concluded that *Fusarium culmorum* had caused the damage by building up on the rhizomes, and also by liberating some toxins during the decay process. The amount of weed root biomass present in the soil when glyphosate is applied seems to be an important factor in this process. The greater the root biomass available as a potential substrate, the higher the number of *Fusarium* propagules that can be produced. At the 2 sites, the overall

number of CFU/g were in the same range, but the maximum difference in CFU/g observed between the control and the sprayed plots was 2 times larger in the field with perennial weeds. Also, more toxins would likely be liberated by the decay process if more biomass was available.

Pest Management Prospective and Conclusion

Baillie *et al.* (1972) tried to create a herbicide that could block the shikimic acid biosynthetic pathway. The basis for their investigation was that such a compound would be safe for animals, since the pathway is present only in plants and in some microorganisms. In addition it could be a good herbicide because this biochemical process is essential in plants. They failed. It is of historical interest that in the same year an efficacious herbicide named glyphosate, selected by the usual empirical mass screening employed by industry, was found to act upon plants via this very same biochemical pathway (Jowarski, 1972). It seems that, by chance, a company found the exemplar herbicide that Baillie *et al.* were systematically looking for. However, it is not certain that glyphosate affects only the shikimic acid pathway; in higher plants, its effect seems to be more complex (Hoagland and Duke, 1982).

Because of all the properties glyphosate has, it is likely that the use of this already popular herbicide will increase. Glyphosate could even change the face of modern agriculture if new crop varieties that are resistant to it come on the market. A resistant alfalfa variety already exists (Brusko, 1983), and the glyphosate-resistant allele has been identified and successfully transferred to *Escherichia coli* (Mig.) Cast. &

Chal. (Stalker *et al.*, 1985). This agrotechnical change raises many problems and questions:

1. If resistant crops can be produced by breeding, it is likely that resistant weeds will emerge.
2. The North American agroecosystem is characterized by monocultures that have a frightfully high level of genetic homogeneity; this new development is likely to increase this level even more.
3. What would be the environmental impacts of using this herbicide on a very large scale?
4. The efficacy of glyphosate varies. If its use is to become more widespread, the instances where the herbicide fails will become more frequent. Therefore, more research on the modes of action will be needed in order to optimize the efficacy of glyphosate. A greater emphasis will have to be put on the research on the effect of plant pathogens as causal agents of glyphosate-induced crop damage, and on their role in the herbicidal action of glyphosate.

This research project has demonstrated that no more damage was done to beans, corn, cucumber, or peas when glyphosate was used for controlling a dense population of annual weeds than when tillage was utilized. At the 2 sites, the major colonizers of representative glyphosate-treated weed species were *Fusarium* spp. It is not known what would be the effect on the crops grown

in soil in which other fungi, e.g. *Pythium* spp., would be the primary colonizers, or where the root biomass would be larger, e.g. in abandoned fields. For cases in which the use of a chemical resulted in an increased incidence of diseases, Altman and Campbell (1977) recommended the use of other techniques to solve the new problem, e.g. another chemical or resistant varieties. I do not agree with this; rather I agree with Griffiths (1981) who recommended, for such cases, to revise the protection program in order to avoid the secondary problem. I believe that the simplest way to avoid herbicide-induced disease injury on crops, would be to find the optimal interval between seeding and spraying. If *Fusarium* spp. enhanced by glyphosate treatment on weeds were shown to be damaging to seeded crops, it should be possible to exploit the fluctuations in *Fusarium* spp. level following treatment in order to minimize crop injury.

Glyphosate can also be used in sylviculture. When applied after formation of final conifer resting buds, and 3-4 weeks before leaf senescence of the brush species to be controlled, it does not affect the conifers (Monsanto, 1983). The effects of glyphosate on root rot organisms should be better understood before any large scale application of this compound is undertaken. For instance, the role of root rot organisms in the herbicidal action of glyphosate when applied in forests should be clarified. It is possible that some colonizers will build up on the brush species to a level that will be detrimental to the conifer crop. In this situation, it is unlikely that *Fusarium*

spp. would be important colonizers since they are uncommon in forest soils (Gordon, 1956; Menzinger *et al.* 1966; Lim *et al.*, 1970). However, *Armillaria* spp., *Phellinus* spp., or *Phoma* spp. are common in forest soils and have wide host ranges; consequently they could cause significant losses. If one wants to study these questions, the first step would be to determine what are the main colonizers of the brush species that have been sprayed with glyphosate. The level of root rot organisms in those stands should be assessed and compared with levels in stands where other brush control techniques have been used. The major challenge in investigating this potential problem is that injuries to the crops could be noticeable only many years after the application of glyphosate.

Glyphosate is a useful herbicide. Its utilization will continue and/or increase. I have demonstrated that the use of this herbicide to control weeds can lead to an increase in soil-borne propagules of *Fusarium* spp. However, before any concrete recommendations that would minimize the potential for herbicide-induced damage can be formulated for the use of glyphosate in agriculture or sylviculture, questions related to host and site specificity and the effects of physical environments variables must be addressed.

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