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EFFECT OF COMBINED AND SPLIT APPLICATIONS OF *PHOMOPSIS CONVOLVULUS* AND DICAMBA ON CONTROL OF FIELD BINDWEED (*CONVOLVULUS ARVENSIS* L.)[1]

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ABSTRACT

Field bindweed (*Convolvulus arvensis* L.) is a serious weed in agricultural areas and its control is difficult with existing weed control strategies. A new potential control method with a fungus, *Phomopsis convolvulus,* used as a bioherbicide, has been suggested. In this study, when the bioherbicide application was combined with the chemical herbicide dicamba, (3,6-dichloro-*o*-anisic acid), weed control was improved at the highest rates of bioherbicide and herbicide. Tank mix application of *P. convolvulus* and dicamba seems to have acted synergistically to increase field bindweed mortality. When *P. convolvulus* was applied one and three days after dicamba as a split application, with the highest rate of dicamba used, lower rate of *P. convolvulus* was even more effective than combined applications.

1. Research project conducted at McGill University, Quebec, Canada, during sabbatical leave of the senior author.
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اثر کاربرد های ترکیبی و جداگانه *Phomopsis convolvulus* و دایکامبا بر کنترل پیچک صحرائی (*Convolvulus arvensis* L.).

حسین غدیری و ا. ک. واتسون

به ترتیب استادیار بخش زراعت واصلاح نباتات دانشکده کشاورزی دانشگاه شیراز واستاد بخش علوم گیاهی، دانشگاه مک گیل، کوبک، کانادا.

چکیده

پیچک صحرایی یکی از علفهای هرز زیانبار در مناطق کشاورزی است و کنترل آن با روش‌های موجود کنترل علفهای هرز مشکل است. یک روش بالقوهٔ کنترل با قارچی بنام *Phomopsis convolvulus*، که به عنوان یک علف کش زیستی مورد استفاده قرار می گیرد، پیشنهاد گردیده است. در این مطالعه، هنگامیکه ترکیب علف کش زیستی فوق با علف کش شیمیایی دایکامبا در بالاترین میزان هر دو بکار برده شد، کنترل مطلوب پیچک صحرایی حاصل گردید. به نظر می رسد که کاربرد مخلوط این دو علف کش زیستی و علف کش شیمیایی بصورت سینرژیستیک باعث افزایش مرگ و میر پیچک صحرایی شده است. هنگامیکه علف کش زیستی فوق، بصورت جداگانه، یک و سه روز پس از کاربرد علف کش شیمیایی دایکامبا مورد استفاده قرار گرفت، در بالاترین میزان مصرف دایکامبا، میزان کمتر علف کش زیستی تاثیر بیشتری در کنترل پیچک صحرایی در مقایسه با کاربرد ترکیبی آن دو داشت.

INTRODUCTION

Field bindweed is an important perennial weed in most provinces of Canada and throughout much of the United States and in most temperate regions of the world (13). This weed is also considered to be a serious weed in most provinces of Iran including Fars province (4,5). It has long been considered a serious weed in agricultural areas and it is most troublesome in cereals, beans, corn (*Zea mays* L.) and orchards (13). Traditional methods of controlling field bindweed, normally combine cultivation and crop rotation with the intensive use of postemergence herbicides (3,14). A new potential control method with a fungus, *Phomopsis convolvulus* Ormeno, has been suggested (11). Under favorable environmental conditions, this foliar pathogen was reported to incite leaf spots and anthracnose lesions on field bindweed thus causing reduced growth and regeneration of field bindweed plants of different ages and resulting in good weed control (9). However, environmental restraints could reduce the efficacy of this pathogen in the field. If additional stresses could be applied to field bindweed plants, *P. convolvulus* could be more effective. Preparations of *P. convolvulus* may have to be modified by adding chemical herbicides, growth regulators, or other additives in order to reliably control field bindweed in the field under a wide range of environmental conditions.

Dicamba is a herbicide that is used for control of both annual and perennial broadleaf weeds in some agronomic and horticultural crops. It causes epinasty of young shoot and proliferative growth in susceptible plants (1,2). Dicamba can reduce the shoot dry weight of field bindweed plants treated with as little as 0.14 kg ai ha^{-1} (unpublished). This stress on field bindweed caused by dicamba applicaton may be ideal for the growth and development of *P. convolvulus*, causing an epidemic and killing field bindweed plants. It could possibly enhance effectiveness of the pathogen in the field or reduce the amount of inoculum required to achieve acceptable control. This paper describes the effect of combined and split applications of dicamba and *P. convolvulus* for field bindweed control.

MATERIALS AND METHODS

Inoculum Production

Development of an efficient inoculum production method is an important aspect of bioherbicide research. *P. convolvulus* was therefore grown on a succession of substrates to maximize production of conidia. Small pieces of agar with mycelium from the stock culture, which consisted of a single-conidium isolate of *P. convolvulus* (8412) grown on potato carrot agar slants in small vials and maintained under mineral oil at 4°C (12) were transferred to half-strength fresh potato dextrose agar (PDA; Difco, Detroit, MI) in petri dishes (9-cm dia.). Cultures were incubated at room temperature (21 ± 1°C) with 12 to 14 h of fluorescent (cool-white) light for 45-60 days. Cultures at this stage had conidial droplets oozing from pycnidia embedded in the mycelial mat. Conidial suspensions were obtained by flushing the surface of sporulating cultures several times with approximately 10 ml of deionized water. One ml of this suspension (approximately 10^8 conidia ml^{-1}) was used to seed 20 g of pot barley

(*Hordeum vulgare* L.) grains, previously added to a 250-ml erlenmeyer flask, moistened with 20 ml of water, and autoclaved for 17 min (100 kPa and 120° C). Culture flasks were kept under room conditions (as described above). To harvest conidia, 50 ml of deionized water were added to each flask, and the flasks were shaken to suspend conidia in water. Contents of each flask were then poured through a soil sieve (250 µm) lined with two layers of cheese cloth. Inoculum density in the filtrate was determined with the aid of a haemocytometer and adjusted to the desired density with water.

Plant Production

Field bindweed seeds (Valley Seed Service, Fresno, CA) were washed and imbibed under warm running tap water for 15 to 16 h. Five germinated seeds were sown in potting medium (Pro-Mix BX,Les Tourbieres Premier Ltee, G.P. 2600, 454 Chemin Temiscouata, Riviere du Loup, Que. G54 4G9) in 10-cm diameter plastic pots and thinned to three seedlings per pot after emergence.

Until time of inoculation, seedlings were grown in controlled-environment chambers (Conviron, Model E-15. Controlled Environments. 1461 St. James Street, Winnipeg, Man. R3H OW9) adjusted to 19/17° C day/ night temperature, a 16-h photoperiod, and light intensity of 250 $\mu E\ m^{-2}\ s^{-1}$. Plants were fertilized weekly with a water-soluble fertilizer (1 g of N:P:K, 20-20-20 l^{-1} of water).

General Inoculation Procedures

Field bindweed plants at three to five leaf stage were inoculated with various densities of conidia suspended in 0.1% (W/V) gelatin (BDH Chemicals, Toronto, Ont.) solution, using a spray chamber (Research

Instrument Manufacturing Co. Ltd., Guelph, Ontario) with a full-cone nozzle (Teejet GTO 0.7, Spraying Systems Co., Wheaton, IL) operated at approximately 200 kPa air pressure, a speed of 0.85 kh, to give the desired spray volume of 500 L ha^{-1}. Inoculated plants were incubated in a dark dew chamber (Percival, Model E-54UDL, Boone, Iowa) at 100% relative humidity and 20°C for 18 h and subsequently transferred to a controlled-environment chamber under the original conditions.

Assessment of Weed Control

Mortality was evaluated for each plant and results were pooled and averaged for each pot. Completely necrotic plants were classified as dead when aerial parts were completely necrotic.

Leaf area of plants was measured using a ΔT leaf area meter (ΔT Devices, Cambridge, England) and recorded as the leaf area of three plants per pot.

Plants were cut at the soil level two weeks after inoculation, dead leaf and stem material discarded, and living tissues were dried in paper bags for 4 to 5 days at 70° C. Roots were carefully removed from the potting media, soaked in water for approximately 5 min, washed in running water, blotted dry with paper towels, and dried in paper bags for 4 to 5 days at 60° C. Dry weights of above-ground and root biomass were recorded separately.

Effect of Tank Mix Combinations of Dicamba and *P. convolvulus* on Field Bindweed

Dicamba at 0, 0.075, 0.15, and 0.3 kg ai ha^{-1} and *P.convolvulus* at 0, 10^7,10^8 and 10^9 conidia m^{-2} were applied in all combinations with each combination having two components, to field bindweed seedlings at the three to five-leaf stage. Treated plants were incubated in the dark dew chamber for 18 h following treatment, and measurements were then recorded as described above. The experiment was repeated.

Effect of Split Applications

Dicamba at 0, 0.075, 0.15, and 0.3 kg ai ha^{-1} was applied to field bindweed seedlings at three to five leaf stage. After one and three days, *P. convolvulus* at 0, 10^7, 10^8 and 10^9 conidia m^{-2} was applied to produce a combination of treatments that included two components. Treated plants were incubated in the dark dew chamber for 18h following *P. convolvulus* treatment, and measurements were then recorded as described above. The experiments were repeated.

A randomized complete block design with four replications was used. Data were analyzed with a factorial analysis of variance (ANOVA). Total sums of squares for effects of dicamba, *P. convolvulus* , and their interaction were broken down into single degree of freedom sums of squares using polynomial regression. Final regression equations were determined using only factors that were determined by the factorial ANOVA to be significant at α=0.05. For the regression analysis, the *P. convolvulus* rate of 0 spores m^{-2} was arbitrarily set at 0.0001 spores m^{-2} (1 spore ha^{-1}) in order that a log value (-4) could be used. Response surfaces were plotted by solving regression equations (generated by using treatment means) only for levels of dicamba and *P. convolvulus* actually tested in the experiments.

RESULTS AND DISCUSSION

Effect of Tank Mix Combinations of Dicamba and *P. convolvulus* on Field Bindweed

P. convolvulus alone at 10^9 conidia m^{-2} resulted in mortality of some plants (8%) while dicamba alone did not kill field bindweed (Fig. 1). The highest level of mortality (76%) occurred with *P. convolvulus* at 10^9 conidia m^{-2} in combination with dicamba at 0.3 kg ai ha^{-1}. Examination of the data suggested that the interaction might have been synergistic; alone the two components caused little or no mortality, while in combination they caused high mortality.

Both dicamba alone and *P. convolvulus* alone reduced the amount of field bindweed above-ground biomass compared with the control (Fig. 2). The high combination resulted in the highest level of weed control. Regarding the effect of *P. convolvulus* and dicamba, their interaction seems to be synergistic.

Both components also reduced leaf area of field bindweed plants compared with the control (Fig. 3). The effect of *P. convolvulus* alone was more severe than that of dicamba alone. The effect of dicamba was observed mainly on the top leaves of the plants. As the rates of both components in combination increased, leaf area of field bindweed severely decreased.

Effect of split applications

When dicamba was applied at the three to five-leaf stage, and *P. convolvulus* was applied one and three days later, weed control was still highest (100%) at the highest rates of both components (Figs. 4,5).

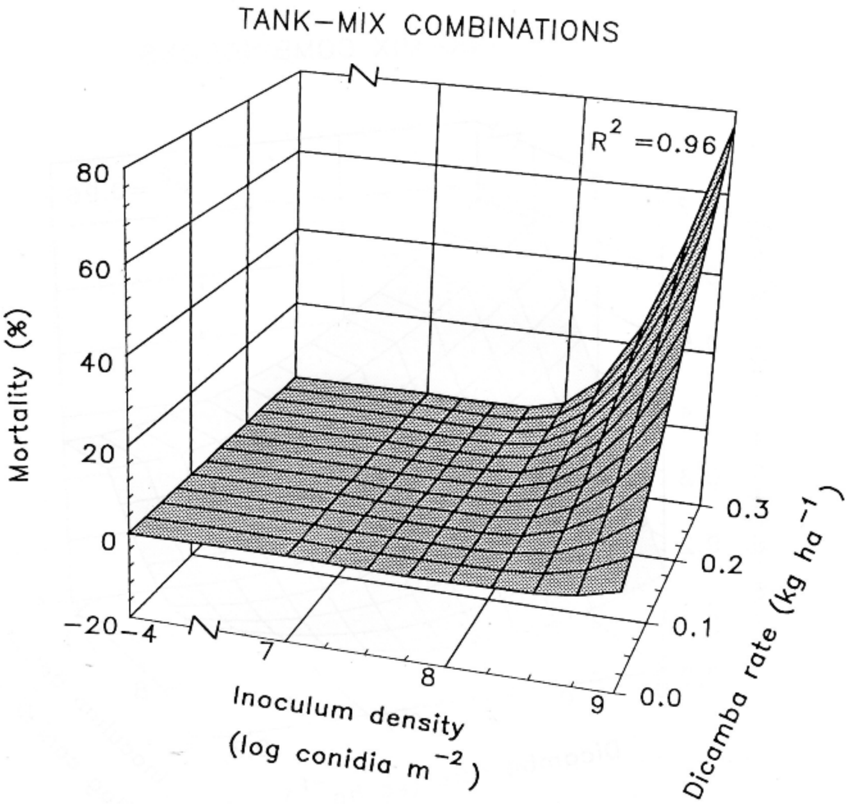

Fig. 1. Response surface for field bindweed mortality for combined application of *P. convolvulus* and dicamba.

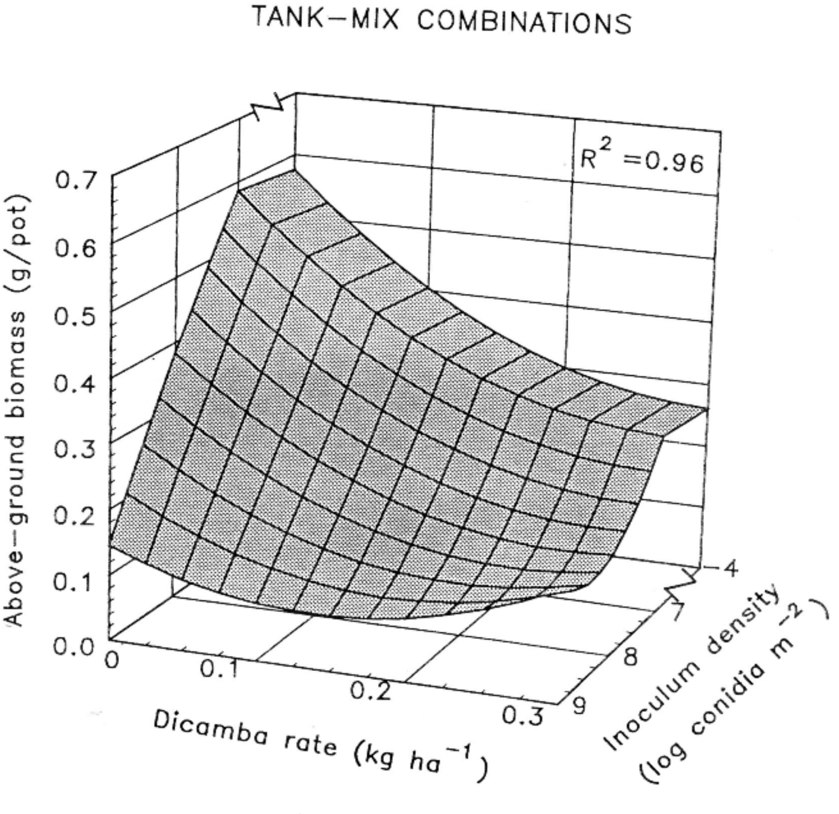

Fig. 2. Response surface for field bindweed above-ground biomass for combined application of *P. convolvulus* and dicamba.

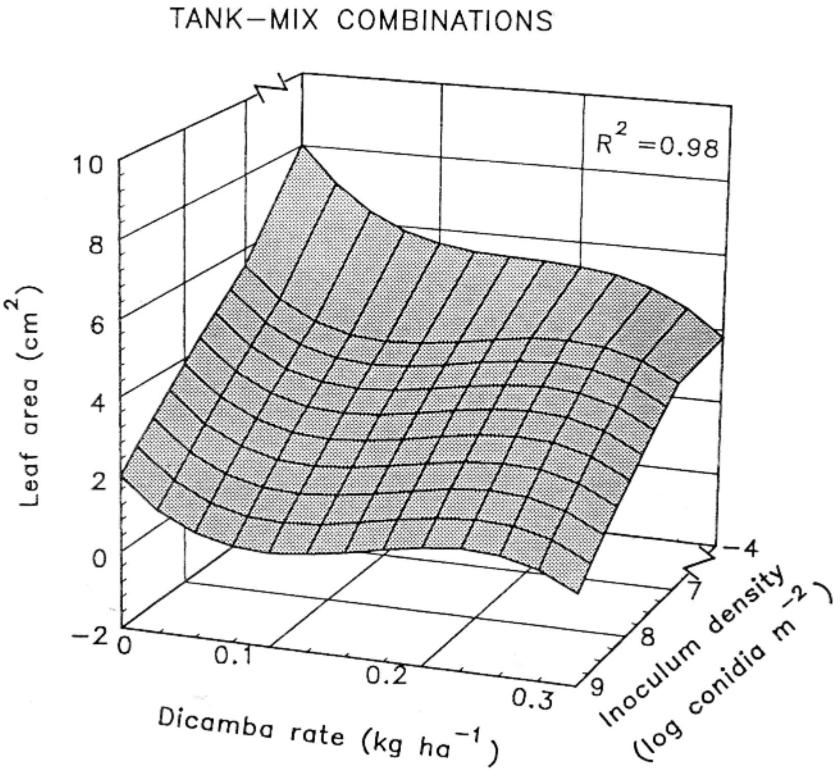

Fig. 3. Response surface for field bindweed leaf area for combined application of *P. convolvulus* and dicamba.

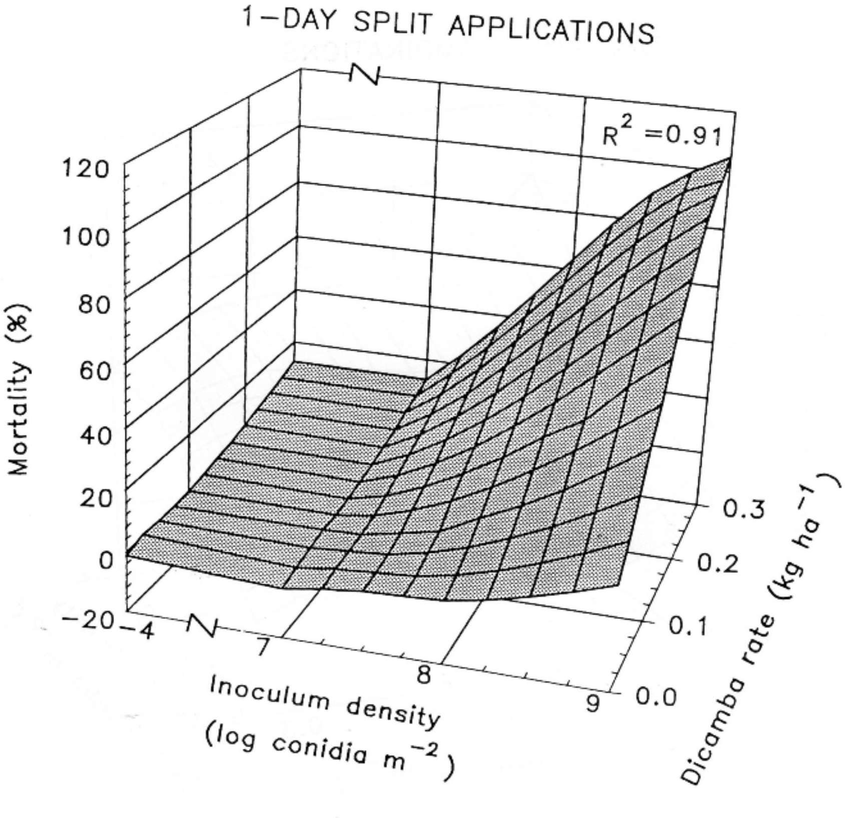

Fig. 4. Response surface for field bindweed mortality for 1-day split application of *P. convolvulus* and dicamba.

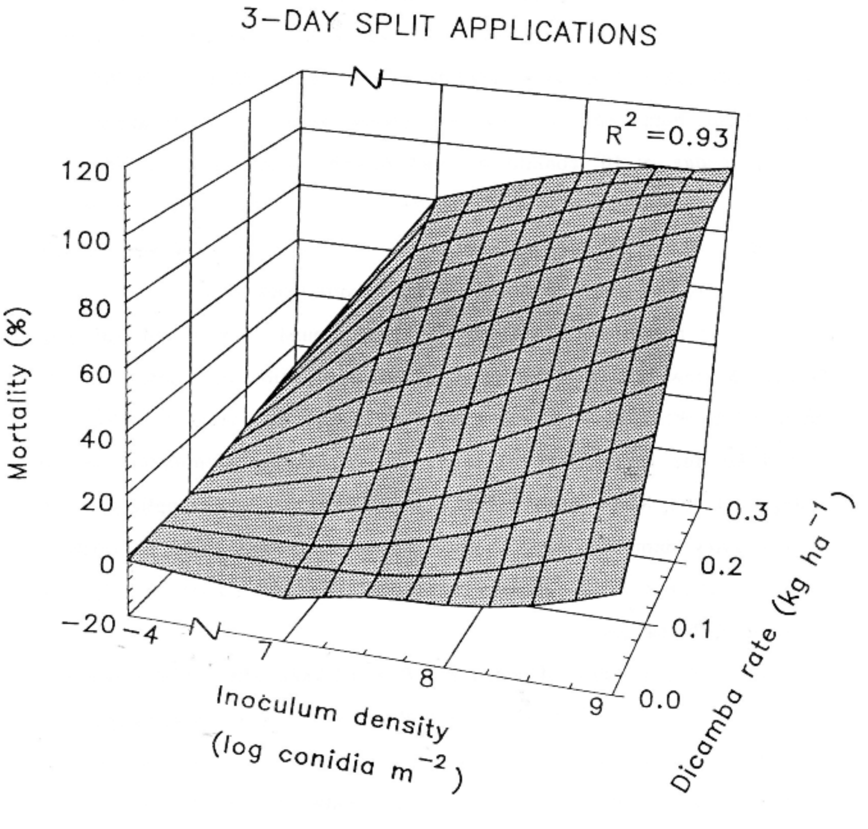

Fig. 5. Response surface for field bindweed mortality for 3-day split application of *P. convolvulus* and dicamba.

However, with the highest rate of dicamba used, lower rate of *P. convolvulus* (10^8) in split applications was more effective than when both components were applied as a tank mix at the three to five- leaf stage. Dicamba at 0.3 kg ha $^{-1}$ might have made field bindweed weak so that subsequent applications of *P. convolvulus* had more influence. In the tank mix, field bindweed mortality was almost zero for combination with *P. convolvulus* at 10^8 conidia m^{-2} and dicamba at 0.3 kg ha^{-1}. When the same rate of dicamba was applied first and the same density of *P. convolvulus* one and three days later, however, control was greatly increased with 60 and 90% mortality, respectively (Figs. 4,5).

Similar results were obtained for above-ground biomass and leaf area data. The low inoculum density as a split application was more effective than when the same rate was applied as a tank mix (Figs. 6,7,8,9). This could be due to the lack of injury during conidial germination of the fungus at the lower rates in split applications (7); split applications at lower rates improved weed control. *In vitro* testing should be done to indicate whether or not dicamba interfered with germination and mycelial growth of the fungus.

Split applications of dicamba and *P. convolvulus* also affected root dry weight of field bindweed. When the highest rate of dicamba was applied at the three to five-leaf stage, and the highest density of *P. convolvulus* was applied one and three days later, field bindweed root dry weight was severely reduced (Figs. 10,11).

Similar studies have demonstrated that a bioherbicide and a chemical herbicide can fit in with a weed management system, either with tank mixing or as split applications (6,7,8,10). In this study, there appeared to be no compatability problems between dicamba and *P. convolvulus* . When

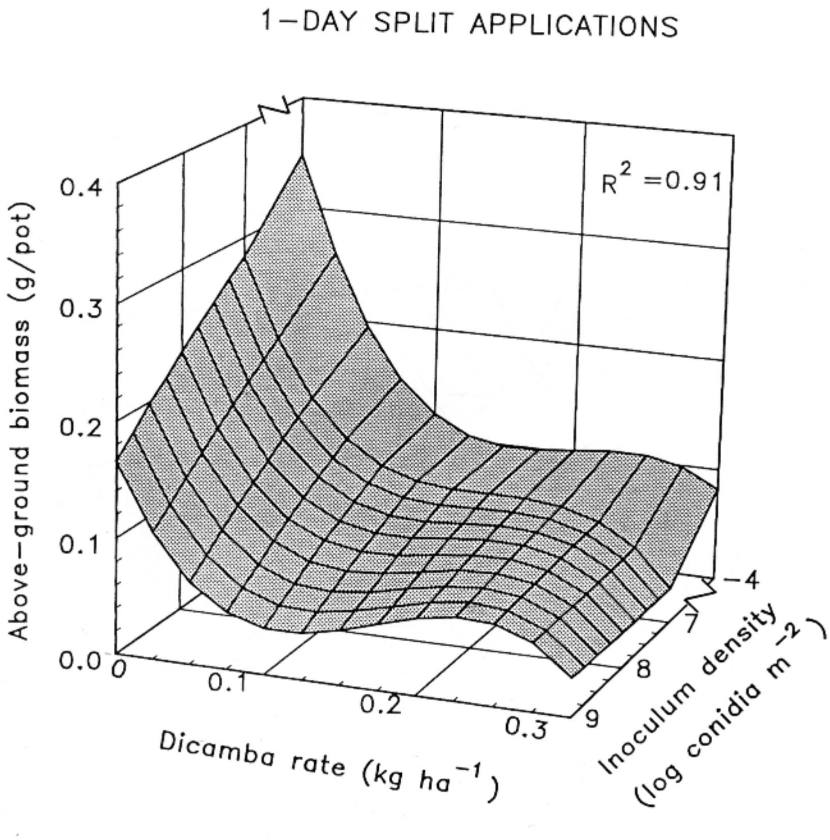

Fig. 6. Response surface for field bindweed above-ground biomass for 1-day split application of *P. convolvulus* and dicamba.

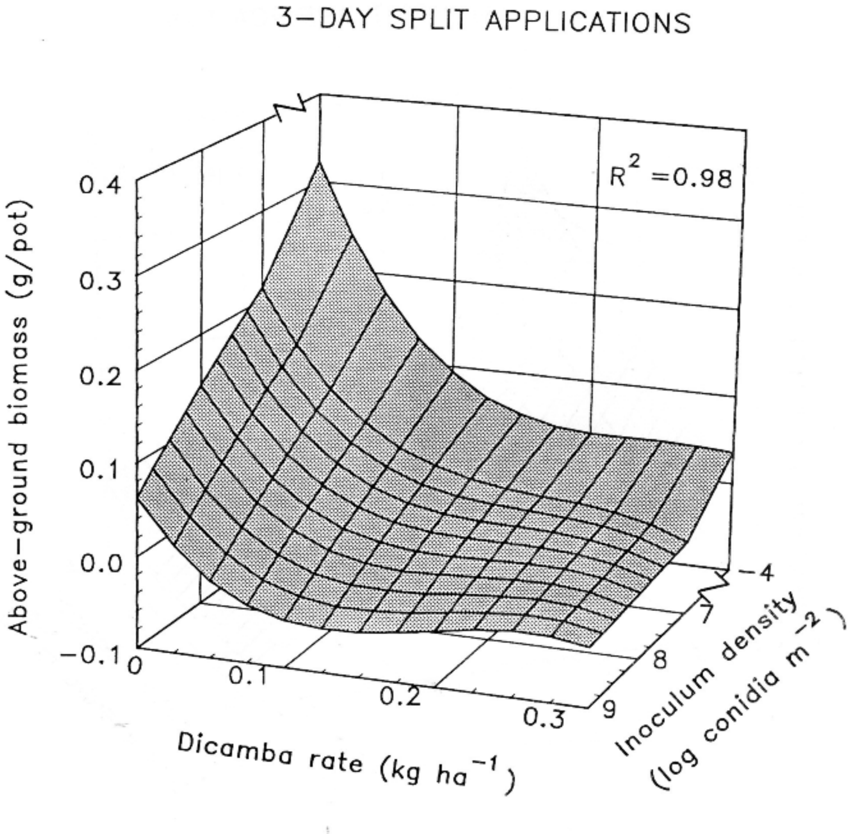

Fig. 7. Response surface for field bindweed above-ground biomass for 3-day split application of *P. convolvulus* and dicamba.

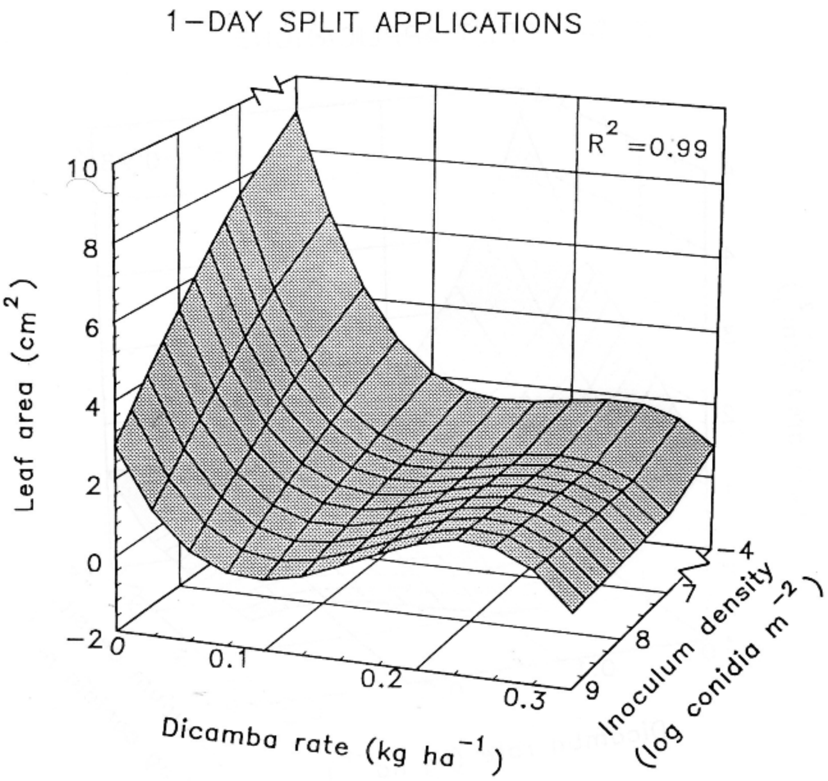

Fig. 8. Response surface for field bindweed leaf area for 1-day split application of *P. convolvulus* and dicamba.

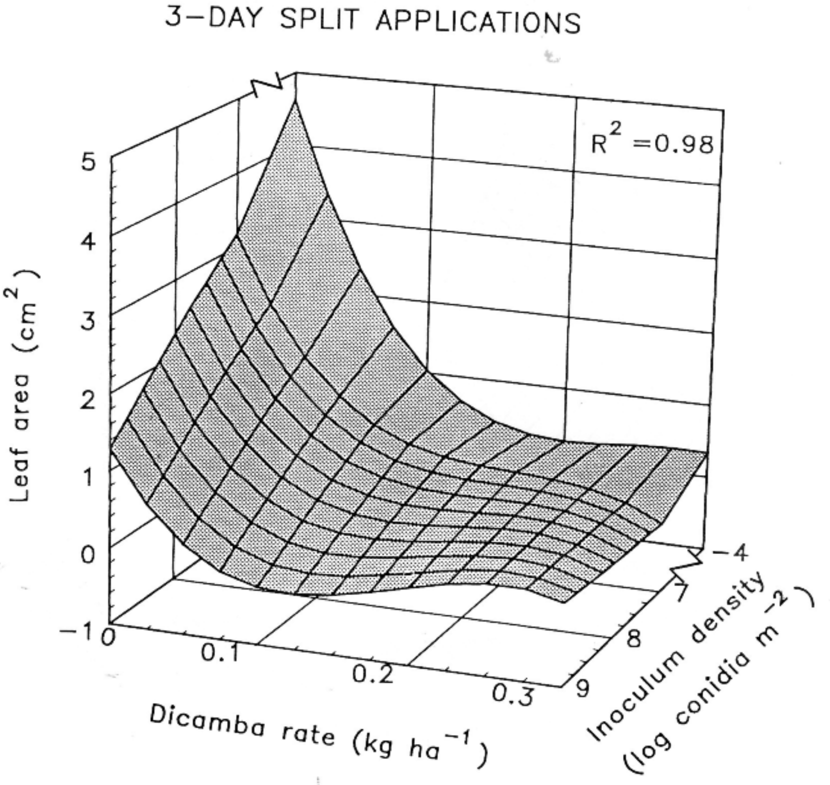

Fig. 9. Response surface for field bindweed leaf area for 3-day split application of *P. convolvulus* and dicamba.

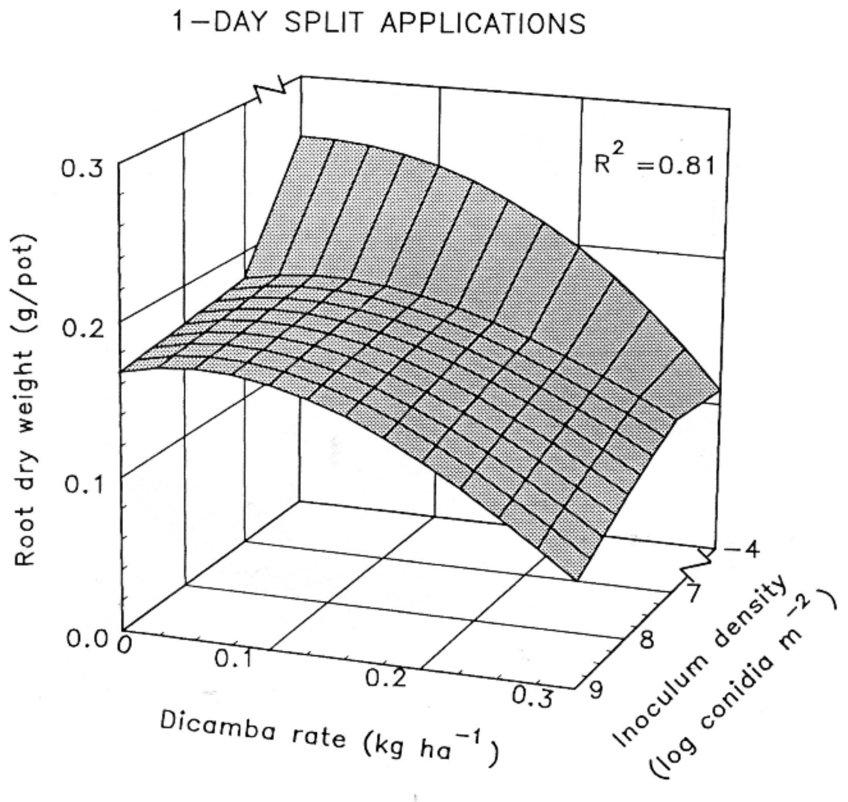

Fig. 10. Response surface for field bindweed root dry weight for 1-day split application of *P. convolvulus* and dicamba.

3-DAY SPLIT APPLICATIONS

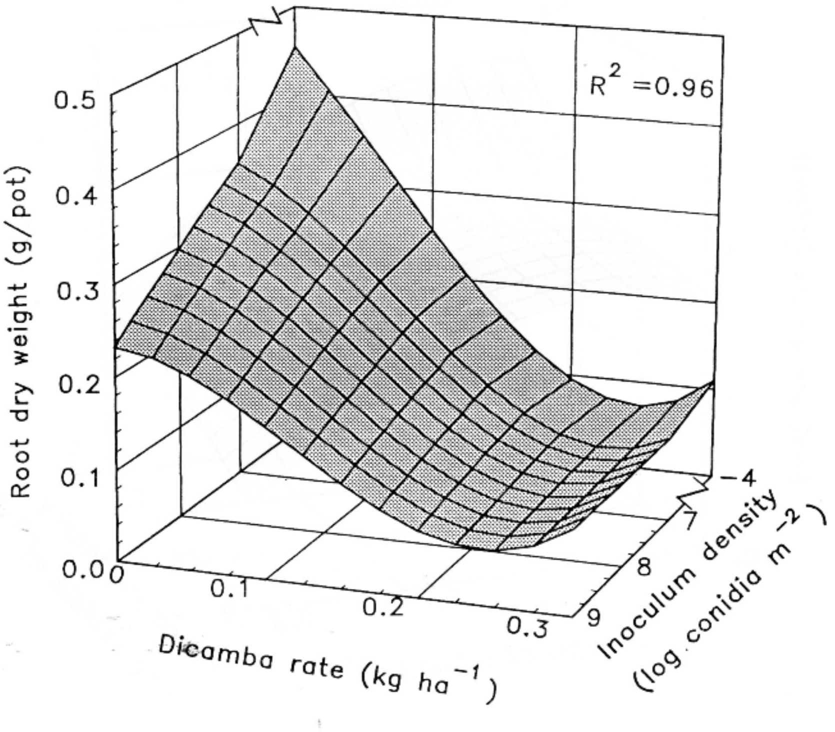

Fig. 11. Response surface for field bindweed root dry weight for 3-day split application of *P. convolvulus* and dicamba.

applications were combined, only the highest rates of dicamba and *P. convolvulus* caused the maximum field bindweed mortality. However, in split applications, the lower rates of both components also caused a great deal of mortality. Therefore, dicamba in split application with *P. convolvulus* appears to have great potential for field bindweed control. Further trials are needed to determine the effectiveness of this appliaction in the field.

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