BIOLOGICAL CONTROL

# Evaluation of Formulation and Volume Application Rate on the Secondary Pick-Up of *Metarhizium acridum* (Driver & Milner) Bischoff, Rehner & Humber Conidia on *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae)

Roberto T Alves[1] & Roy P Bateman[2]

[1]*Embrapa Cerrados, BR 020 km 18, Planaltina, DF, CEP 73310-970, Brasil, Email: roberto.alves@embrapa.br;*

[2]*The International Pesticide Application Research Centre, Silwood Park, Buckhurst Road, Ascot, Berks, SL5 7PY, United Kingdom, Email: r.bateman@imperial.ac.uk*

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Avaliação da Formulação e do Volume de Aplicação na Eficiência do Resíduo da Pulverização de Conídios de *Metarhizium acridum* (Driver & Milner) Bischoff, Rehner & Humber, em *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae)

RESUMO - Os efeitos do resíduo da pulverização de conídios de *Metarhizium acridum* (Driver & Milner) Bischoff, Rehner & Humber, formulados em óleo adjuvante emulsionável e em uma mistura de óleos minerais, sobre a mortalidade de adultos de gafanhotos, *Schistocerca gregaria* (Forskål), foram avaliados. Os efeitos do volume de aplicação (VAR) e a presença de conídios aplicados por um atomizador de disco rotativo, sobre as características de pulverização daquelas duas formulações também foram avaliados. As formulações de conídios foram aplicadas sobre plântulas de trigo por um pulverizador de trilho projetado para simular aplicações de campo em ultra-baixo volume, mas em condições de laboratório. Não se observou diferença significativa entre as duas formulações e entre os quatro volumes de aplicação sobre a sobrevivência de *S. gregaria*, mas houveram diferenças nos tempos médios de sobrevivência (AST) de *S. gregaria* entre as doses. Quando a dose aumentou o AST diminuiu. Estudos sobre as características de pulverização revelaram que, quando o VAR aumentou, o diâmetro do volume mediano e o número de gotas/cm$^2$ também aumentou. Ambas as formulações apresentaram uma distribuição de gotas uniforme com características de aplicação de gotas controladas. Quando o VAR aumentou, o volume das gotas também aumentou, o número de conídios/gota diminuiu e o número de conídios/cm$^2$ também diminuiu. A formulação em óleo adjuvante emulsionável foi tão eficiente quanto a formulação em óleos puros nessas condições experimentais e demonstraram um grande potencial para o controle biológico de insetos-praga, quando pulverizada com a técnica e equipamento apropriados onde as condições ambientais são favoráveis.

PALAVRAS-CHAVE - micoinseticidas, controle de gafanhotos, aplicação de gotas controladas.

ABSTRACT - Effects of the spray residue of *Metarhizium acridum* (Driver & Milner) Bischoff, Rehner & Humber, formulated in emulsifiable adjuvant oil and in a mixture of mineral oils, on the mortality of the desert locust adults, *Schistocerca gregaria* (Forskål), were evaluated. The effects of volume application rates (VARs) and the presence of conidia applied by a spinning disc atomiser, on the spray characteristics of those two kinds of formulations were also evaluated. The conidial formulations were applied to seedlings of wheat by a track sprayer designed to simulate ultra-low volume field spraying in laboratory conditions. No significant differences between the two formulations and between the four

VARs on the proportional survival of *S. gregaria* were revealed, but there were significant differences between doses. The average survival times (AST) of *Schistocerca gregaria* were calculated and there were significant differences only between doses. When the dose increased, the AST of the insect decreased. Studies on spray characteristics revealed that when the VAR increased, the droplet volume median diameter and the number of droplets/cm$^2$ also increased. Both formulations gave uniform droplet distributions with characteristics of controlled droplet application sprays. When the VAR increased, the droplet volume also increased, the number of conidia/droplet decreased and the number of conidia/cm$^2$ decreased. The emulsifiable adjuvant oil formulation worked as well as the oil formulation in these experimental conditions, and it has a great potential to control insect pests, when sprayed with the appropriate equipment and technique, where the environmental conditions are suitable.

KEY WORDS - mycoinsecticide, locust control, controlled droplet application.

The control of insect pests using entomopathogenic fungi to avoid chemical applications and to increase environmental protection is a desirable option (Ahmed & Leather 1994). For a safe and an optimally targeted application with the development of the inherent biological efficacy of the product, the fungal conidia have to be properly formulated.

The addition of 10% of an emulsifiable oil enhanced infectivity of *Metarhizium anisopliae* (Metsch.) Sorokin in water-based formulation to yellow meal worm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) (Alves *et al*. 1998).

Infections can arise from direct impaction of spray droplets, secondary pick-up of conidia from the spray residue and via horizontal transmission of the pathogen from infected individuals (Bateman *et al*. 1998).

Alves *et al*. (2002) showed that emulsifiable adjuvant oil formulations of *M. anisopliae* var. *acridum* can be used to formulate and store conidia for medium-term and probably for long-term under cooled conditions similarly to oil-based formulations.

Faria *et al*. (2007) showed that oil-based formulation of *M. anisopliae* var. *acridum* can be used for *Rhammatocerus schistocercoides* Rehn (Orthoptera: Acrididae) control in Mato Grosso Brazilian State. They obtained a grasshopper control of 68%, applying controlled droplet application techniques in field conditions.

The main purpose of this investigation was to evaluate the effects of the spray residue of *Metarhizium acridum* (Driver & Milner) Bischoff, Rehner & Humber formulations sprayed at different volume application rates and doses by an experimental track sprayer, on the mortality of desert locust, *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae).

Effects of the volume application rate and presence of *M. acridum* conidia, sprayed by a spinning disc atomiser, on the spray characteristics of an emulsifiable oil-based formulation and an oil-based formulation were also evaluated.

## Material and Methods

### Effects of formulation and volume application rate on the secondary pick-up of *Metarhizium acridum* conidia pathogenic to the desert locust *Schistocerca gregaria*

The desert locust, *S. gregaria,* was reared in cages inside a controlled environment (CE) room (50±5% R.H. and 27±1ºC).

Conidia of *M. acridum* isolate IMI 330189 were formulated in water plus 10% of emulsifiable oil and in a mixture of two paraffinic oils (Table 1).

Conidial concentrations of both formulations were then calibrated using a Neubauer's chamber, to be applied at the equivalent doses and proportional volumes per hectare (Table 2).

Both control formulations with no conidia were also sprayed at 10 l/ha (blank formulations). The fungal formulations were applied on seedlings of wheat by a track sprayer designed to simulate field spraying in laboratory conditions (Bateman, 1994).

After each application, 20 adult locusts were placed into a sprayed cage with wheat and held for a period of 100 minutes in a CE room at 30±0.5 ºC, 30±5% RH with a day:night regime of 14:10 h, to have contact with the spray residue of the *M. acridum* formulations. The insects were then housed in plastic boxes and incubated in the same CE room. Mortality

Table 1. Trade names, composition, and suppliers of the emulsifiable oil and mineral oils.

| Trade name | Composition | Company |
|---|---|---|
| Codacide® | Emulsifiable oil containing 95% rapeseed oil and 5% of emulsifiers | Microcide Ltd. |
| Ondina EL | Refined paraffinic oil | Shell Oil Co. |
| Shellsol T | Refined paraffinic oil | Alcohols Ltd. |

Table 2. Conidial number of *Metarhizium acridum* per millilitre applied to obtain the equivalent dose at different volume application rates.

| Equivalent doses (conidia/ha) | Equivalent volume application rates (l/ha) | | | |
|---|---|---|---|---|
| | 0.3 | 1.0 | 3.2 | 10.0 |
| $10^{10}$ | $3.13 \times 10^7$ | $1 \times 10^7$ | $3.13 \times 10^6$ | $1 \times 10^6$ |
| $10^{11}$ | $3.13 \times 10^8$ | $1 \times 10^8$ | $3.13 \times 10^7$ | $1 \times 10^7$ |
| $10^{12}$ | $3.13 \times 10^9$ | $1 \times 10^9$ | $3.13 \times 10^8$ | $1 \times 10^8$ |

was recorded daily during 14 days.

The experiment was a factorial design with two formulations, four volume application rates and three doses of conidia. There were controls for each formulation and three replicates with 20 insects each carried out on three different occasions. Factorial analyses of variance (ANOVA) of the survived insect results were performed.

**Effects of the volume application rate and presence of *Metarhizium acridum* conidia on the spray characteristics of an emulsifiable oil-based and an oil-based formulations**

Spraying samples of each treatment were collected to evaluate the number of droplets per cm$^2$ by water and oil-sensitive paper.

The droplet size spectra were assessed by spraying the formulations with a spinning disc atomiser fitted in the cabinet of a Malvern® Particle Size Analyzer to analyse the influence of both formulations without and with conidia ($10^{12}$ conidia/ha) on droplet size, droplet distribution and droplet volume.

Volume median diameter (VMD) and relative Span are two useful parameters for describing droplet size spectra (Matthews, 1992). VMD is the diameter at which half of the volume of spray contains droplets larger than the VMD (in µm) while the other half has smaller droplets (Matthews, 1992). A range of droplet size can be indicated by the relative Span which is calculated from the volume distribution only. Span is calculated using the formula:

$$\text{Span} = (D_{(v,0.9)} - D_{(v,0.1)}) / D_{(v,0.5)}$$

A Span of less than 1.0 is characteristic of a controlled droplet application spray (Bateman, 1993).

The droplet volume, expressed in picolitres (pl) was calculated and based on the volume represented by the VMD (Bateman *et al*., 1998). After that, the number of conidia per droplet was calculated and based on the droplet volume, equivalent VAR and on the dose applied ($10^{12}$ conidia/ha). Finally, the number of conidia per cm$^2$ was calculated timing the number of conidia per droplet by the number of droplets per cm$^2$.

Data factorial analyses of variances (ANOVA) of VMD, Span, droplet volume, number of conidia per droplet, number of droplets per cm$^2$ and number of conidia per cm$^2$ were performed using the statistical package SPSS® for Windows™.

Table 3. Mean proportional survival of the desert locust, *Schistocerca gregaria* infected by the spray residue of two conidial formulations applied on wheat seedlings under different doses, after 14 days.

| Doses (conidia/ha) | Mean proportional survival with standard error | | |
|---|---|---|---|
| | water plus 10% Codacide® | 50% Shellsol T plus 50% Ondina EL | Pooled mean |
| $10^{10}$ | 0.83 ± 0.04 a | 0.82 ± 0.05 a | 0.82 ± 0.03 a |
| $10^{11}$ | 0.62 ± 0.05 b | 0.63 ± 0.05 b | 0.63 ± 0.03 b |
| $10^{12}$ | 0.21 ± 0.06 c | 0.15 ± 0.03 c | 0.18 ± 0.03 c |

Means followed by the same small letter within the same column are not significantly different ($p$<0.05).

## Results

**Effects of formulation and volume application rate on the secondary pick-up of *Metarhizium acridum* conidia pathogenic to the desert locust *Schistocerca gregaria***

The results of ANOVA revealed no significant differences between the two formulations and between the four VARs on the mean corrected proportional survival of *S. gregaria*, after 14 days, but there were significant differences between doses (df = 2, 71; F = 65.14; $p$<0.05) (Table 3). There were no significant interactions between the main factors of formulations, VARs and doses.

The insects started to die five days after treatment for both formulations (Figure 1 and 2). These results were very similar to the results obtained by Bateman *et al*. (1998) using large semi-permanent cages in Niger, Africa. There was a tendency for the emulsifiable oil formulation to work better at the VARs of 3.2 and 10 l/ha (Figure 1). On the other hand, there was a tendency for the oil-based formulation to work better at 0.3 and 1 l/ha (Figure 2).

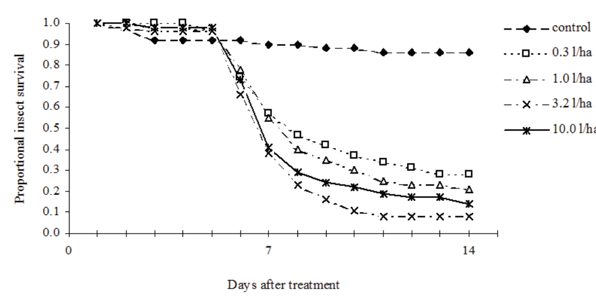


Figure 1. Proportional survival of the desert locust, *Schistocerca gregaria*, infected by the spray residue of *Metarhizium acridum* formulated in water plus 10 % Codacide® at the dose of $10^{12}$ conidia/ha and sprayed at different volume application rates.

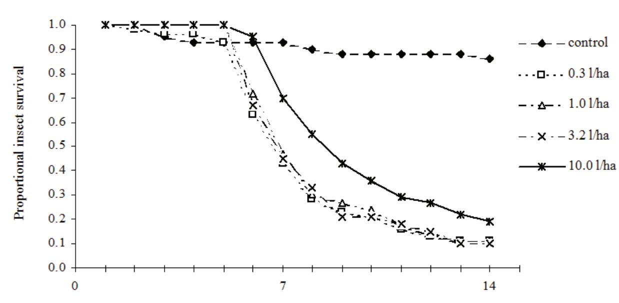


Figure 2. Proportional survival of the desert locust, *Schistocerca gregaria*, infected by the spray residue of *Metarhizium acridum* formulated in 50 % Shellsol T plus 50 % Ondina EL at the dose of $10^{12}$ conidia/ha and sprayed at different volume application rates.

Table 4. Effects of the equivalent volume application rate on the spray characteristics of an emulsifiable oil-based formulation and an oil-based formulation.

| Formulation | Flow rate (ml/min) | Peristaltic pump speed setting | Equivalent VAR (l/ha) | VMD with standard error (µm) | Relative Span with standard error | Estimated droplet volume with standard error (pl) | Estimated number of conidia per droplet with standard error |
|---|---|---|---|---|---|---|---|
| Water plus 10% Codacide | 0.5 | 9 | 0.3 | 87.69 ± 20.18 bc | 2.36 ± 1.24 a | 471.44 ± 299.59 ab | 1473.26 ± 936.23 a |
| | 1.5 | 30 | 1.0 | 77.35 ± 0.40 c | 0.40 ± 0.04 c | 242.35 ± 3.77 b | 242.35 ± 3.77 bc |
| | 4.8 | 94 | 3.2 | 107.63 ± 2.62 ab | 0.63 ± 0.11 c | 655.13 ± 46.63 a | 204.73 ± 14.57 bc |
| | 15.1 | 99 | 10.0 | 110.49 ± 3.48 a | 0.70 ± 0.10 c | 710.44 ± 65.38 a | 71.04 ± 6.54 c |
| 50% Shellsol T plus 50% Ondina EL | 0.5 | 14 | 0.3 | 67.91 ± 4.22 c | 4.43 ± 0.66 b | 167.87 ± 32.57 b | 524.58 ± 101.77 ab |
| | 1.5 | 45 | 1.0 | 73.48± 0.75 c | 0.33 ± 0.10 c | 207.83 ± 6.32 b | 207.83 ± 6.32 bc |
| | 4.8 | 71 | 3.2 | 75.19± 0.70 c | 0.49 ± 0.13 c | 222.66 ± 6.22 b | 69.58 ± 1.94 c |
| | 15.1 | 90 | 10.0 | 80.81± 1.52 c | 0.75 ± 0.02 c | 273.82 ± 15.42 b | 27.38 ± 1.54 c |

Means followed by the same small letter within the same column are not significantly different ($p$<0.05).

**Effects of the volume application rate and presence of *Metarhizium acridum* conidia on the spray characteristics of an emulsifiable oil-based formulation and an oil-based formulation**

ANOVA on VMD revealed significant differences between formulations and between VARs formulations, but there were no significant differences between droplet sizes with and without conidia.

There were significant differences between VARs for the relative Span (Table 4). Droplet volume was significantly affected by formulations and VARs (Table 4).

The number of conidia per droplet was significantly affected by VARs. It depends on the droplet volume and the dose applied (Table 4).

The number of droplets/cm$^2$ was significantly affected by formulations and VARs. When the VAR increased, the number of droplets/cm$^2$ also increased (Figure 3).

The locusts were infected by an estimated number of conidia per cm$^2$ (Figure 4) on the wheat seedlings and it was significantly affected by formulations.

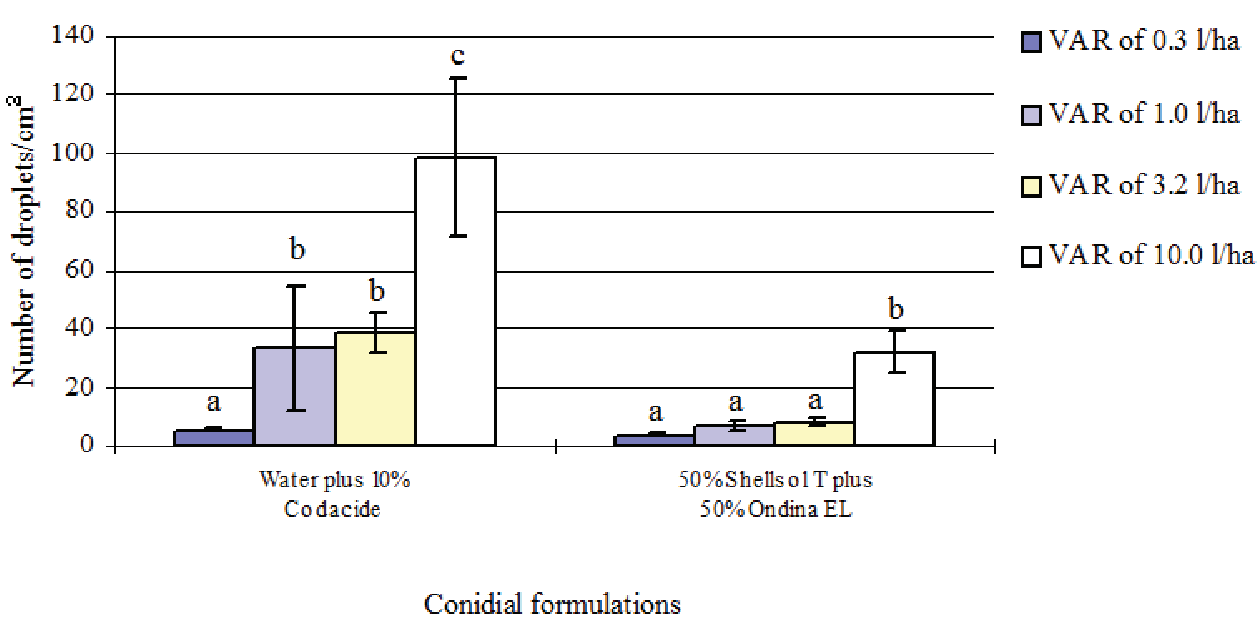


Figure 3. Number of droplets/cm$^2$ (± s.e.) of two conidial formulations sprayed at different equivalent VARs with a spinning disc atomiser working at 6030 rpm and counted on water and oil-sensitive papers. Note: Means followed by the same letter within the same formulation are not significantly different ($p$<0.05).

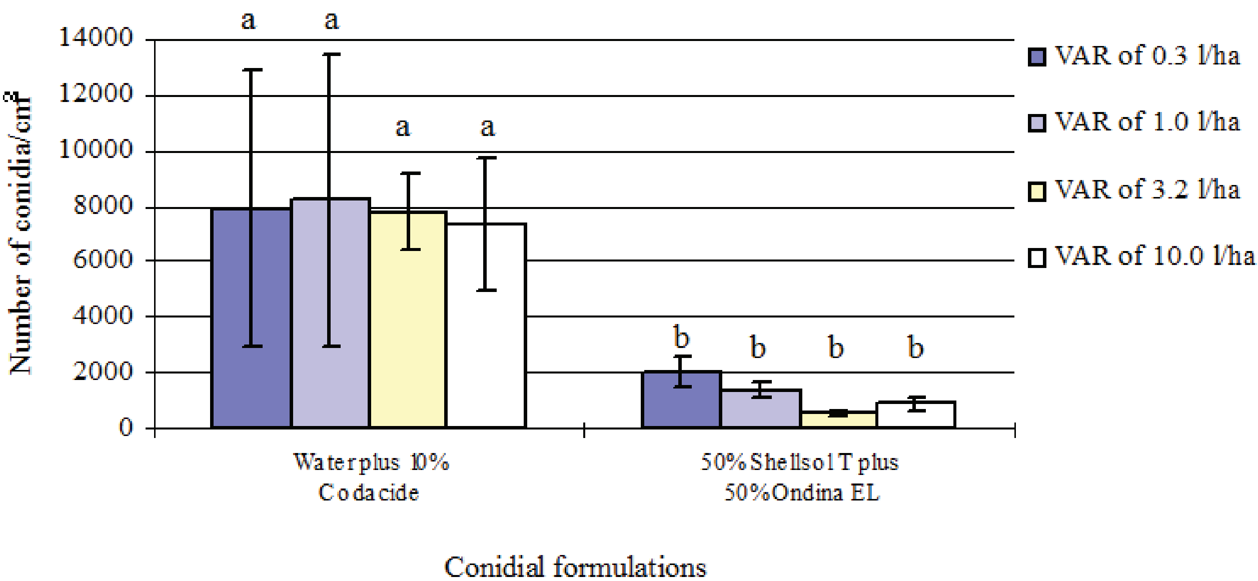


Figure 4. Estimated mean number of conidia/cm$^2$ (± s.e.) of two conidial formulations sprayed at different equivalent VARs with a spinning disc atomiser working at 6030 rpm. Note: Means followed by the same letter are not significantly different ($p$<0.05).

**Discussion**

The insects were not hit directly, but they became infected by the spray residue of the conidial formulation which had impacted on the foliage.

The most effective dose was $10^{12}$ conidia/ha for both formulations and this dose is very similar to the dose applied in the field by different authors (Bateman *et al*., 1994; Lomer *et al*., 1993). Therefore, the spray residue on foliage had an amount of conidia sufficient to infect and to reduce the desert locust population in the experimental conditions by secondary pick-up.

Oil-based formulations are, economically and technically, appropriate to be sprayed with rotary atomisers at ultra-low VARs (below 5 l/ha). They can be broken into a very large number of small droplets with a good distribution because they are more viscous and less volatile than conventional water-based formulations. This could explain why the oil-based formulation tended to work better at the three lowest VARs than at 10 l/ha and the emulsifiable oil-based formulation tended to work better at the three highest VARs than at 0.3 l/ha, mainly seven days after treatment.

The emulsifiable oil conidial formulation presented the VMD for all equivalent VARs in the right size-band 75-150 µm suitable for water-based controlled droplet application (CDA). The oil-based formulation also had all droplet sizes in the appropriate size-band 50-100 m (Bateman, 1993) for all equivalent VARs. These results can help to explain why there were not significant differences between the proportional survivals of *S. gregaria*, when the formulations were applied at different VARs.

Both conidial formulations presented uniform droplet distributions with characteristics of CDA sprays when applied at 1, 3.2 and 10 l/ha. A good droplet coverage on foliage is very important to control insect pests in the field. Locusts are very mobile insects and maybe this was another reason why all VARs with different Spans have worked equally at the same doses.

About the droplet density, the peristaltic pump was sending more liquid for high VARs than for low VARs, but the spinning disc atomiser was working at the same rotation per minute (6030) all the time. Then, the liquid from an equivalent VAR, 10 l/ha, was broken into a number of droplets higher than for other, smaller VARs for both formulations. The difference between formulations could be caused by the difference of quality between the water-sensitive paper and oil-sensitive paper. The water-sensitive paper is more easily sensitised by water-based formulations than the oil-sensitive paper by oil-based formulations, but comparisons between the number droplets/cm$^2$ within the same formulations could be done without problems.

The number of conidia per cm$^2$, for a given dose, is dependent on the number of droplets per cm$^2$ and on the number of conidia per droplet. When the VAR increased, the number of droplets per cm$^2$ increased and the number of conidia per droplet decreased. At the end, the number of conidia per cm$^2$ was not significantly different within the same formulation when applied at different VARs.

In field conditions, it could be more effective to spray the oil-based conidial formulation at 1 l/ha VAR because it was effective in controlling the insect pest at this VAR and, at the same time, it presented uniform droplet distribution with characteristics of CDA sprays. For the emulsifiable oil conidial formulation, the application could be more effective enlarging the droplet size using the VAR of 3.2 l/ha to allow some evaporation of water during transport of the spray droplets to the target area and to increase the droplet settling with a reduction of the time in flight. This could be reinforced by the tendency of the two formulations to work better at those VARs and to present uniform droplet distributions with characteristics of CDA sprays.

The emulsifiable adjuvant oil conidial formulation worked as well as the oil-based formulation in these experimental conditions, and it has a great potential to control insect pests, when sprayed with the appropriate equipment and technique, particularly in areas where the environmental conditions are suitable.

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