VII.5 Lab Studies and Field Trials With the Fungus *Beauveria bassiana* Against Grasshoppers

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More than 150 years ago, the Hyphomycete fungus *Beauveria bassiana* was recognized as the cause of a disease fatal to insects (Steinhaus 1967). *B. bassiana* is a common insect pathogen (an agent that causes disease) found on all continents except Antarctica (Humber 1992). Hundreds of isolates of the fungus, including five from grasshoppers, are listed in the U.S. Department of Agriculture (USDA) collection of Entomopathogenic Fungal Cultures (Humber 1992).

In the fungus' life cycle, conidia (spores) adhere to the grasshopper cuticle (part of the exoskeleton). The conidia germinate, and the germ tube penetrates the cuticle. The fungi replicate inside the insect haemocoel (body cavity) in the form of blastospores (spores produced by a budding process). Degradative enzymes destroy the internal structures of the grasshopper.

When in sufficient quantity, the fungus causes sickness within 3 days. The grasshopper reduces its feeding and becomes immobile. Typically, infected grasshoppers die between 4 and 10 days after infection depending on their species, age, and size, and the dose of conidia. After death, under conditions of high humidity, blastospores form hyphae (filaments of the vegetative structure of the fungus) that emerge through the insect's cuticle, sporulate (produce spores), and cover the insect in a characteristic white growth (fig. VII.5–1).

Figure VII.5–1—An immature rangeland grasshopper, *Melanoplus sanguinipes*, exhibits the fungus *Beauveria bassiana*, which caused its death. (Photo by K. Christian Reuter.)

In 1987, Mycotech Corporation in Butte, MT, isolated, from an infected grasshopper found in Montana, a strain of *Beauveria bassiana* that is virulent (disease-causing) to several grasshopper species in laboratory bioassays. Since that time, Mycotech has developed and refined production capabilities to the point that large-scale commercialization is planned upon the final development of an acceptable formulation for delivering the pathogen to grasshoppers in the field. The following summarizes some of the research conducted since early 1991 in the development of formulations of *Beauveria bassiana* usable against grasshoppers on rangeland.

Laboratory Studies, 1991–93

During this period, we conducted more than 20 different replicated studies. The objectives provided for (1) developing equipment and procedures for our laboratory studies, (2) studying the effect of *Beauveria bassiana* on different age groups of grasshoppers, (3) comparing of formulations, and (4) comparing the virulence of different batches of commercially produced *B. bassiana*.

Test formulations were sprayed from a tower apparatus in the lab to simulate aerially applied sprays (fig. VII.5–2). Applications were conducted according to a detailed standard operating procedure (Foster and Reuter 1991 unpubl.). Laboratory-reared *Melanoplus sanguinipes* grasshoppers supplied by South Dakota State University were used for all studies. All tests focus on a dose of 1×10^{13} (1 trillion) spores/acre as a standard. Depending on the specific test protocol, we sprayed grasshoppers and/or live vegetation upon which the grasshoppers were to be confined.

When grasshoppers were sprayed, third instars through adult stages were sprayed singly or in groups consisting of from 5 to 20 grasshoppers per group. After spraying, the grasshoppers were monitored daily for death, usually for 2 weeks. In tests where grasshoppers were sprayed, fresh food was provided to surviving grasshoppers daily, and dead grasshoppers were held singly under high humidity conditions for observance of sporulation.

Figure VII.5–2—Spray tower used to simulate aerially applied sprays for bioassaying grasshoppers in the laboratory. (APHIS photo by Lonnie Black.)

Initial studies demonstrated the superiority of an oil formulation over a water formulation. A typical example of results from one of these tests is shown in figure VII.5–3. In later studies where candidate field formulations were compared, we focused primarily on different oil types with various additives selected for ultraviolet light protection and emulsion stabilization (formulation stability). Two petroleum oils performed equally well as base carriers; however, one is significantly less expensive. We found that formulations involving emulsifiable concentrates tend to be more difficult to spray consistently in the laboratory. However, our results indicate that such compounds may provide higher mortality in field application.

In studies where untreated grasshoppers were confined on sprayed vegetation, we showed a significant decrease in mortality on vegetation that had been exposed to sunlight for longer than 24 hours (fig. VII.5–4). However, two formulations currently under development show promise for extending protection beyond 24 hours.

Third-, fourth-, and fifth-instar grasshoppers were easily infected and very susceptible to sprays equivalent to 1×10^{13} spores/gal/acre. However, compared to these results, two separate studies with adult grasshoppers showed a greatly reduced level of mortality at the same dose. Subsequent studies in which adults with amputated wings were sprayed showed that reduced mortality in adults cannot be attributed to physical protection provided by wings, which shield a major portion of the abdomen from the spray.

We conducted several studies to compare spores from different productions and to evaluate shelf life. Spores stored in oil for up to 1 year performed as well as dry conidia powder stored for an equal period. A 1992 spring production as well as a new isolate both performed similarly to spores produced in 1991. However, a 1992 fall production sampled resulted in some inconsistencies during the physical spraying. Slightly cooler temperatures during the spray operation may have affected the sprayability of the formulation. Also, a new harvesting method at the production facility resulted in some larger particles of spore powder, increasing spray problems.

Field Studies—1991

A 9-acre rangeland plot near Edgemont, SD, infested with predominantly second- and third-instar grasshoppers of mixed species, was aerially sprayed with an oil formulation containing 8×10^{12} spores/gal/acre (fig. VII.5–5). Grasshopper moralities measured in this plot were compared to a similar untreated adjacent plot (Foster et al. 1991 unpubl.).

We evaluated mortality on six grasshopper species by collecting grasshoppers from both plots after application and confining them in (1) small rearing cups (fig. VII.5–6), which we moved to the laboratory for daily monitoring, and (2) bottomless field cages (fig. VII.5–7) estab-

lished after treatment in both plots. Additionally, 0.1-m^2 rings (Onsager and Henry 1977) were used to delimit counting areas for estimating total field populations of grasshoppers.

Beauveria bassiana caused mortality in all six species of the grasshoppers tested. Both grasshoppers held in rearing cups in the laboratory and those caged on native vegetation in the field demonstrated significant mortality in treated populations compared to untreated populations. Some species were killed faster than others, but we do not know if this is due to inherent susceptibility or behavioral differences between the species.

In rearing cups, the average reduction of all species combined in treated populations was about 96 percent at 8 days after treatment. Mortality in the controls during the same period was about 34 percent. In field cages, the mean reduction of all species combined was 79 percent and 11 percent for treated and untreated populations, respectively, at 9 or 10 days after treatment.

In field plots, counts of unconfined populations in treated and untreated plots showed average differences in mortality that ranged from about 39 percent to 63 percent at 3 to 15 days after treatment (fig. VII.5–8).

We also used field cages to determine the general manner in which grasshoppers pick up the spores. Immediately after application, grasshoppers from the untreated plots were collected and caged in the treated area to determine pickup through feeding activity. Treated grasshoppers were caged in the untreated plot to determine the mortality associated with direct contact. Treated grasshoppers were caged in the treated plot to determine the total mortality, and untreated grasshoppers were caged in the untreated plot as a control.

At 11 days after treatment, there were no significant differences in grasshopper mortality between the direct deposition, feeding activity, or combined direct deposition/feeding activity treatments. All three treatments showed significantly greater mortality than the untreated

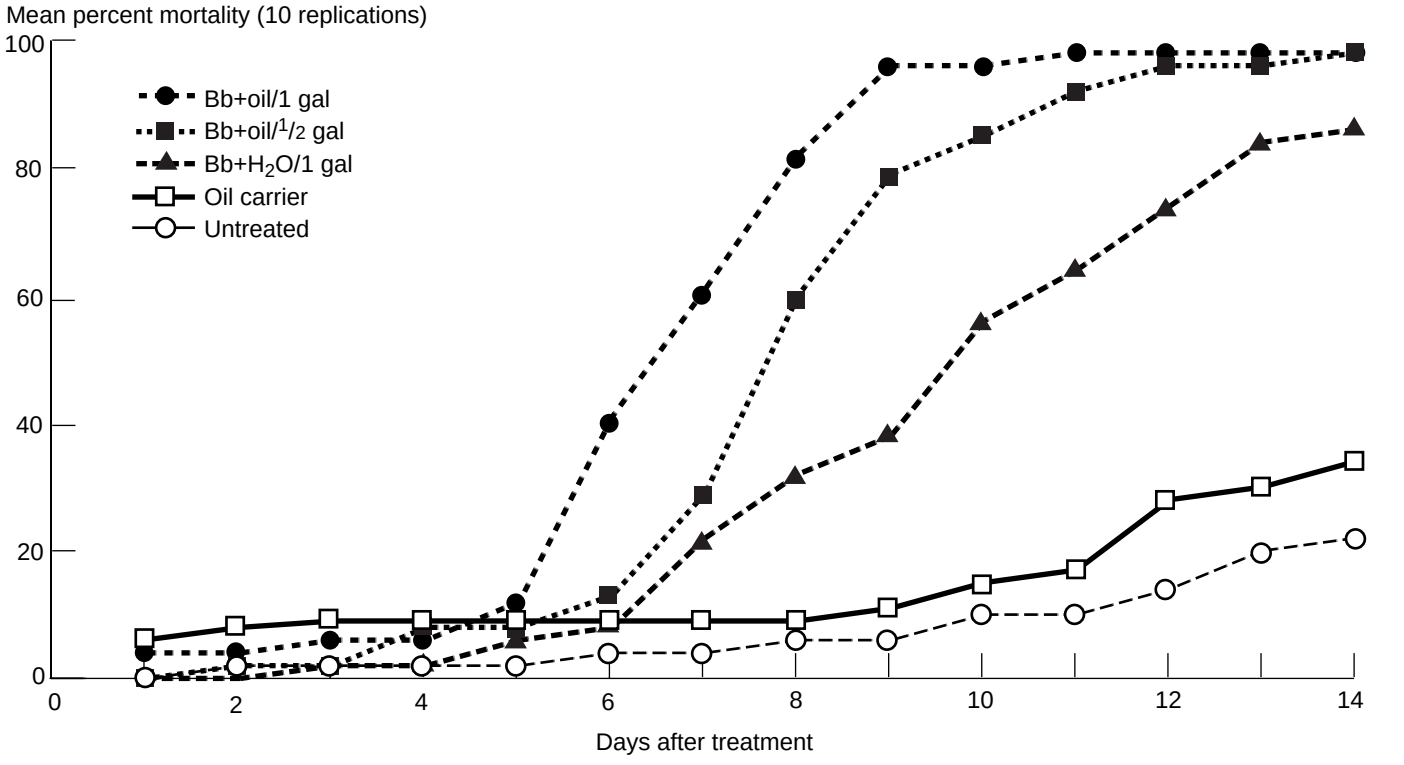

Figure VII.5–3—Mortality of caged grasshoppers treated with experimental formulations of *Beauveria bassiana* at 1×10^{13} conidia per acre.

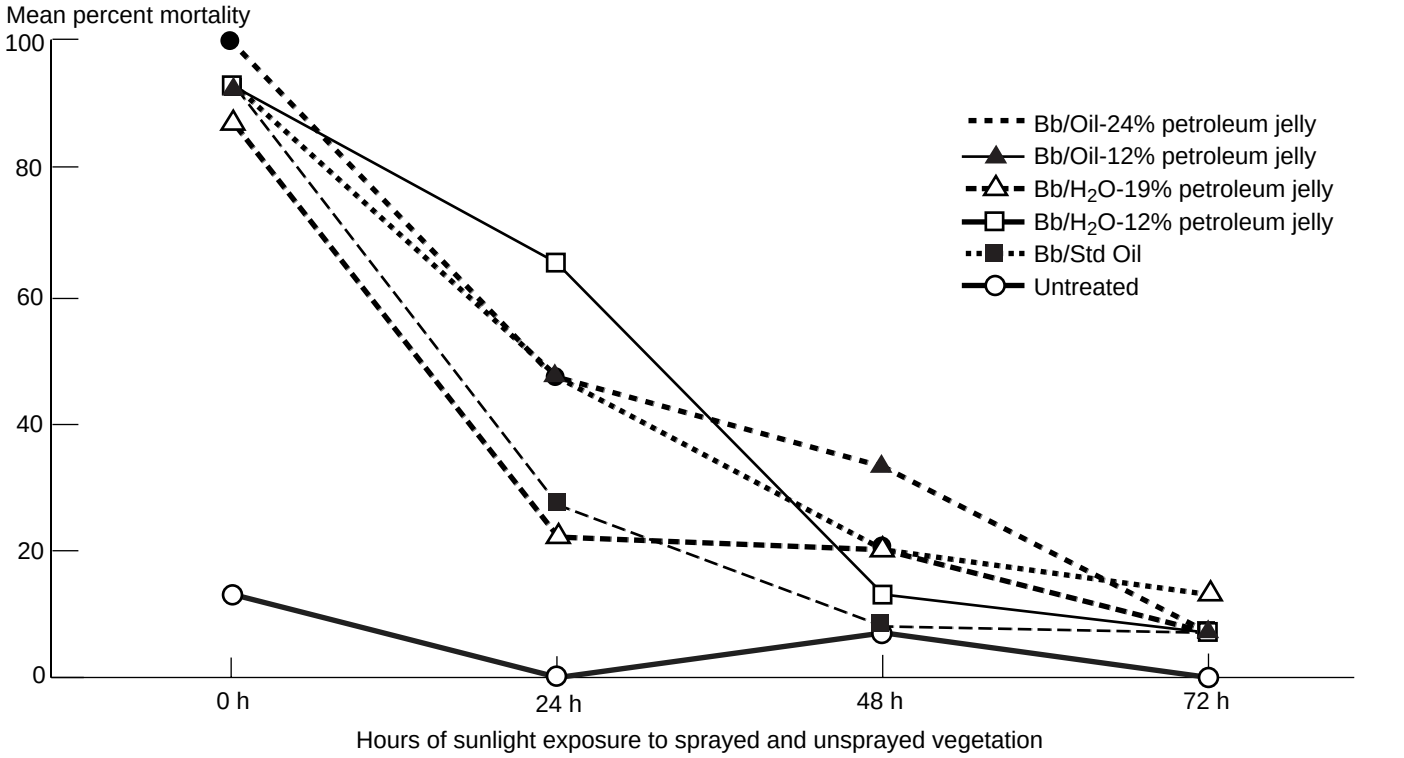

Figure VII.5–4—Effect of grass treated with selected formulations of *Beauveria bassiana* and exposed to several periods of sunlight on grasshopper survival after 9 days. All treatments were applied at a volume of 1 gal/acre containing 1×10^{13} spores.

Figure VII.5–5—The first aerial application of the fungus *Beauveria bassiana* was applied at 1 gal/acre to a rangeland plot near Edgemont, SD in 1991. (Photo by Cliff Bradley.)

Figure VII.5–6—Four-ounce rearing cups used to confine test grasshoppers after they have been treated. (APHIS photo by R. Nelson Foster.)

check. Our data indicate that pickup may occur through either direct impingement (direct striking by spray droplet) or feeding activity. We do not know if the feeding activity component is simply due to contact with the mouthparts of the grasshopper during feeding or actual ingestion of spores.

We evaluated the short-term residual activity of the spores by caging untreated grasshoppers approximately 10 hours after treatment in the treated plot. Survival of the conidia on vegetation was evaluated in the sprayed plot by taking vegetation samples at three posttreatment intervals. These samples were washed, diluted, and placed on selective agar plates, where fungus colonies developed from each colony-forming unit. The colonies then were counted to estimate the number of viable (living) conidia.

Figure VII.5–7—Bottomless field cages used to confine test grasshoppers in the field are inspected carefully to determine the daily insect mortality. (APHIS photo by R. Nelson Foster.)

Figure VII.5–8—Mortality of unconfined field populations of grasshoppers is estimated by counting grasshoppers in metal rings. (APHIS photo by R. Nelson Foster.)

Untreated grasshoppers exposed to the treated vegetation in the field approximately 10 hours after application died at about 3.3 times the mortality rate of untreated grasshoppers over the same period of time, 11 days. The delayed exposure demonstrates the infectivity of spores at least 10 hours after field application and indicates that, in field situations, at least several hours are available for a grasshopper to become infected with the fungus. Results of the study to determine survival of conidia on vegetation in the field showed relatively uniform coverage in the plot and indicated no loss of activity over at least the first 10 hours after application.

Field Studies—1992

Three adjoining 9-acre rangeland plots near Amidon, ND, infested with predominately fourth- and fifth-instar grasshoppers of mixed species were the basis for studies in 1992. One plot was aerially sprayed with 9.5×10^{12} spores/64 oz/acre. One plot was sprayed with 64 oz/acre of the oil carrier (without spores), and the other plot was left untreated for comparison (Foster et al. 1992 unpubl.).

Mortality evaluations were conducted as in 1991, by confining, after treatment, the six predominant grasshopper species in cages held in the laboratory or in the field. The methods used for maintaining the cages and confirming fungus-induced death by sporulation were similar to those employed in 1991. Reduction in the total field population was again estimated by using 0.1-m^2 rings to delimit counting areas.

In this study, the aerial application of *B. bassiana* resulted in substantial mortality of all six species of grasshoppers evaluated. Both grasshoppers held in rearing cups in the laboratory and those caged on native vegetation in the field demonstrated significant mortality in fungus-treated populations compared to untreated populations and populations treated with oil only. These results were generally similar to those obtained in 1991, and again time to mortality varied among species, begin-

ning in as little as 3 days for some species and as much as 4 to 6 days for other species. These differences may be attributed to individual species susceptibility or a result of behavioral avoidance, which limits physical exposure of individual species to direct impingement of the spray droplet.

In rearing cages, the mean reduction of all species combined in treated populations was 95 percent at 8 days after treatment. During the same time period, mortality in the untreated population and the population treated only with oil was 10 percent and 4 percent, respectively. Three species common to both the 1991 and 1992 studies demonstrated very similar responses to the aerially applied *B. bassiana* treatment.

In field cages, the mean reduction for 5 of the 6 species confined in treated populations was 91 percent at 15 to 17 days following treatment. This reduction compared to mortality during the same period in the untreated population and the population treated only with oil of 23 percent and 11 percent, respectively. The sixth species in the study was reduced much quicker: 100-percent mortality occurred by the eleventh day. Its counterparts in the untreated plots and the plots treated with oil showed 26 percent and 16 percent reduction during the same period.

Comparisons of the in-field posttreatment population estimates in single, small plots are difficult to interpret. High densities of grasshoppers, sparse vegetation, small plot size, and local movement all contribute to confounding estimates of nonrestricted in-field populations. Compared to 1991, in-field mortality was lower in this study. In 1992, apparent mortality at 9 days after treatment was only about 20 percent. We did note that vegetation in the 1992 study was much sparser than in the 1991 study and may have offered the spores less protection from sunlight. Using large field plots in future studies should reduce many of the difficulties commonly encountered when comparisons of in-field grasshopper populations on rangeland are attempted.

Field Studies—1993

We focused studies for the first time in 1993 on larger plots than previously used (Foster et al. 1993 unpubl.). That year, we aerially sprayed 24 adjoining 40-acre rangeland plots located near Amidon, ND, infested with predominantly second-, third-, and fourth-instar stages of grasshoppers of mixed species. Two formulations of *Beauveria bassiana* spores were each applied to eight plots. One treatment consisted of 9.9×10^{12} spores/64 oz/acre in an oil formulation, and the other treatment consisted of 9.4×10^{12} spores/64 oz/acre in an oil plus additive (adjuvant) formulation. An oil-only treatment was applied at 64 oz/acre to four plots. Carbaryl was sprayed at 20 oz/acre (0.5 lb/active ingredient [AI] per acre) to four plots as a standard treatment for comparison. Four plots were left untreated to determine the natural changes in the grasshopper population and for comparison with all applied treatments.

In field populations, estimates were again made using 0.1-m^2 rings. A monitoring site located near the center of each 40-acre plot consisted of 40 rings arranged in a circle with rings separated by 5 paces. Field cages were placed adjacent to the ring site in each plot after the treatment was sprayed. Sprayed grasshoppers of two of the dominant species were confined in these cages in a manner similar to that employed in 1991 and 1992 field studies.

Additional field cages were set up in each fungus- and oil-only treated plot and in the untreated plots. These cages were used to study the residual activity of *Beauveria bassiana* over a 5-day period after treatment. Untreated grasshoppers were confined in some cages on the day of treatment and on each of the 5 days following treatment.

Unfortunately, the study's value was lessened by measurable rain (heavy at times) that occurred on 9 of the 13 days that population estimates were made. During the entire study, measurable rain was recorded on 15 of 21 days.

Although incomplete, analysis of counts from rings to date shows that the carbaryl standard was statistically superior to all other treatments at each of the posttreatment interval readings. Good performance of carbaryl under these conditions was expected and is consistent with two of our previous studies where carbaryl was used (Foster et al. 1991 unpubl. and Foster et al. 1993 unpubl.). All other experimental treatments (including the untreated checks) showed erratic results, undoubtedly

confounded by the weather conditions experienced during the study, and were statistically inseparable.

Results from the field cages for the two species studied at 15 days after treatment indicated that both fungus treatments and the carbaryl treatment produced mortality significantly greater than what occurred in the untreated populations. However, mortality in the field cages was somewhat lower than in 1991 and 1992 for the one species that was common to studies in all 3 years.

Residual activity was evident only during the day of treatment. Beyond 1 day, no significant differences in mortality were detected between fungus-treated or untreated grasshoppers.

Under the conditions of this study, evaluations of unproven formulations are confounding and inconclusive at best. However, there is no doubt that carbaryl performed well under these conditions and that the current formulation of *Beauveria bassiana* will need to be improved if it is to be employed under these conditions, or excluded from use under such conditions. Additional replicated studies to obtain information on the original objectives of the 1993 field study and new formulation evaluations are planned for the future.

Summary of Additional Foreign Studies

During the past 5 years, Mycotech has been working to develop fungal pathogens of locusts and grasshoppers for use in integrated pest management (IPM) programs in Africa. This work is in collaboration with Montana State University, the U.S. Agency for International Development, and several African government agencies. These efforts were undertaken to devise alternatives to chemical grasshopper/locust control measures commonly used in Africa. Fungi can fit well into an IPM scheme because they provide control alternatives where chemical insecticides are inappropriate. In fact, because of their relatively slow action, fungi will work best as part of a continuous pest-control strategy, where they can be applied before populations are able to reach damaging levels.

A Mycotech strain of the fungus *Beauveria bassiana* has been tested against grasshoppers and locusts in several small-plot field trials in the west African countries of Cape Verde and Mali. Fungal spores were applied at a rate of 1×10^{13} per acre. Low-volume application of an oil-based formulation (27 ounces to 2 quarts per acre) was made with hand-held spinning disc sprayers. High-volume application of an emulsifiable formulation (2–10 gal/acre) was made with motorized or hand-pumped backpack sprayers. Spores were also formulated on wheat bran bait with a molasses sticker.

In all trials, 80 to 100 percent of treated, caged insects died from *Beauveria bassiana* infection after 7 days. More significantly, replicated 5-acre blocks in Cape Verde, treated with either oil-formulated or emulsion-formulated fungus, showed approximately 50 percent population density reductions measured in the field after 7 days. It is quite encouraging that the insect population in these tests consisted primarily of older nymphs and adults, which have demonstrated more resistance to the fungus in laboratory bioassays.

Mycotech and Montana State University have taken part in an expedition to Madagascar to collect new fungal pathogens of locusts and grasshoppers. The fungi isolated from infected insects are presently being examined for virulence, target specificity, production characteristics, and impact on mammals. The government of Madagascar is particularly interested in using fungi to treat locust populations before the insects expand out of their recessionary (nonoutbreak) areas. When a suitable fungus is identified, field trials will begin.

These promising results indicate that fungal insecticides may be able to play an important role in grasshopper/locust control. This field experience in the harsh African conditions will continue to yield information valuable to the development of fungal insecticides for North America.

Summary and Conclusion

A strain of the entomopathogenic fungus *Beauveria bassiana* has been isolated from U.S. grasshoppers by Mycotech Corporation. Development of mass production capabilities with a potential for large-scale commercialization has resulted in extensive testing of the commercially produced fungus for use against grasshoppers and

locusts. Laboratory studies have demonstrated the insecticidal value of the fungus against several species of grasshoppers and locusts. In 1991, 1992, and 1993, we conducted field studies using cages to demonstrate successful control of several species of confined grasshoppers in the United States when liquid formulations of *Beauveria bassiana* were aerially applied with conventional commercial application equipment. Results of field studies with unconfined grasshoppers in this country are inconclusive to date. Foreign field studies on unconfined populations showed good potential for providing control. Results from the last 3 years suggest the potential for controlling several species of grasshoppers and locusts using a liquid formulation of *B. bassiana*, as a bioinsecticide, and applied with conventional aerial application equipment.

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