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Insecticide Dips for Control of Japanese Beetle and Other Soil-Infesting White Grubs in B&B Nursery Stock[1]

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Abstract

Studies conducted in 1996, 1998 and 1999 show that immersion of root balls in Dursban® (chlorpyrifos) significantly reduces the number of white grubs, particularly Japanese beetle, *Popillia japonica* Newman, in B&B (ball and burlap) nursery stock. In 1996, the influence of root ball size, soil type, and dip time on the efficacy of Dursban® 50WP, Dursban® 4E, and Oftanol® 2F (isofenphos) for white grub control in dipped B&B nursery stock was evaluated. The grubs found within the root balls were primarily oriental beetle, *Exomala orientalis* (Waterhouse), European chafer, *Rhizotrogus majalis* (Razoumowsky), Asiatic garden beetle, *Maladera castanea* (Arrow), northern masked chafer, *Cyclocephala borealis* Arrow and Japanese beetle. With one exception, all the insecticide treatments caused similar grub mortality (96–100%) in 61.0 and 81.3 cm (24 and 32 in) root balls, respectively, and in sand or clay soil. There were no significant differences in grub survival due to dip time (1, 2, and 5 min). However, there were consistently fewer live grubs recovered from the root balls dipped 2 or 5 min compared with the number of live grubs recovered from root balls dipped for 1 min. In 1998, trees with 30.5, 45.7 and 61.0 cm (12, 18 and 24 in) root balls were dipped in either 0.453 or 0.906 kg ai/378.5 liter (1 or 2 lbs ai/100 gal) of Dursban® 4E for 2 mins. Both rates of Dursban® provided 100% control of Japanese beetle grubs in all three root ball sizes. Five trees from each treatment were planted one week after dipping and were evaluated for phytotoxicity up to 12 months after dipping. Most of the trees with 30.5 cm (12 in) root balls dipped in Dursban® died. Three of five trees with 45.7 (18 in) root balls dipped in the low rate of Dursban® and all five root balls dipped in the high rate had small leaves. One tree dipped in the low rate and 2 trees dipped in the high rate died 12 months after dipping. All of the trees with 61.0 cm (24 in) root balls dipped in the low rate of Dursban® survived and showed no signs of phytotoxicity. Trees with the same root ball size dipped in the high rate of Dursban® exhibited smaller leaves in 3 of the 5 trees. In 1999, trees with 61.0 cm (24 in) root balls were dipped in either 0.453, 0.226 or 0.113 kg ai/378.5 liter (1, 0.5 or 0.25 lbs ai/100 gal) of Dursban® 4E for 2 mins. All rates of Dursban® provided 100% control of Japanese beetle grubs.

Index words: Japanese beetle, scarab, chlorpyrifos, grub, quarantine.

Significance to the Nursery Industry

Shipment of nursery stock infested with Japanese beetle grubs can introduce the beetle to new areas. Most unifested states maintain quarantines or certification requirements to reduce the risk of introduction. The USDA, the National Plant Board, and the regulated industry support the continuing harmonization of Japanese beetle quarantine and certification requirements to assure that the pest risks are acceptably managed and to facilitate the orderly marketing of nursery stock in a manner consistent with the National Plant Board Plant Quarantine, Nursery Inspection, and Certification Guidelines (3). Dipping nursery stock in Dursban® (chlorpyrifos) is the only acceptable option that allows growers to treat nursery stock within 3 days of shipping. Although dipping nursery stock can be difficult, messy and potentially hazardous because of the large volume of insecticide required, previous research has shown Dursban® (chlorpyrifos) dips (the only product currently registered for this use) to be consistently effective at reducing white grub infestations in root balls. Current research has demonstrated that dipping B&B (ball and burlap) nursery stock with root balls 61.0 cm (24 in), for 2 min in Dursban® at 0.113 kg ai/378.5 liter (0.25 lb ai/100 gal), which is 1/8 of the recommended rate, effectively controlled white grubs. This study confirmed that dipping root balls of B&B nursery stock in Dursban® can be an effective regulatory treatment for minimizing unnatural dispersal of Japanese beetle and potentially other white grub species.

Introduction

The Japanese beetle, *Popillia japonica* (Newman), a significant pest of nursery crops, feeds on more than 300 species of trees, shrubs and other plants (2). Grubs reside in the soil feeding on the roots of grasses and weeds, but may also consume the young roots of woody ornamental plants (5). Since its discovery in the United States in 1916, the Japanese beetle has spread rapidly and now is established in most of the eastern United States. Potential dispersal of Japanese beetle is of great concern to regulatory officials in many states. In some regions, intensive irrigation and agricultural production have increased the amount of suitable habitat for colonization by Japanese beetles. Japanese beetles can be artificially moved in several ways, including shipment of nursery stock infested with grubs. Although the federal quarantine for nursery stock was terminated in 1979, many states require treatment of nursery stock originating in areas infested with Japanese beetle prior to receiving shipment of that nursery stock.

The goal of the U.S. Domestic Japanese Beetle Harmonization Plan is to minimize the pest risk associated with movement of Japanese beetle host commodities from infested to non-infested areas of the United States. At the 1998 National

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Plant Board meeting, state regulatory officials from all but three states in attendance agreed to comply with the plan. The harmonization plan established four regulatory strategies based on a state's classification (Categories 1–4). Category 1 (primarily the western states) is considered uninfested/quarantine pest, Category 2 (primarily the midwestern states) is uninfested or partially infested/regulated non-quarantine pest, Category 3 (primarily the eastern states) is partially or generally infested/no regulatory significance, and Category 4 (Wyoming) is historically not known to be infested/no regulatory significance.

Currently, the only acceptable treatment for B&B nursery stock sent from a Japanese beetle infested area to Category 1 states is a dip treatment of chlorpyrifos at a rate of 0.906 kg ai/378.5 liter (2 lb ai/100 gal) for root balls 30.5 cm (12 in) or smaller. The chlorpyrifos dip treatment is one of two acceptable treatments allowed for B&B nursery stock [81.3 cm (32 in) or smaller] shipped to Category 2 states from infested areas. The other treatment is the soil application of imidacloprid (Marathon® 60WP or 1G) applied just prior to oviposition or while the grubs or young and actively feeding. The chlorpyrifos dip treatment is the only B&B treatment that can be done shortly before digging and shipping (3 days).

Although dipping is one of few options available to growers, there is little scientific data to support this treatment, particularly with larger root balls [> 30.5 cm (12 in)]. In 1995, Nielsen and Klein (4) conducted a test in which 38.1–45.7 cm (15–18 in) root balls of lilac, *Syringa vulgaris* L., and witch hazel, *Hamamelis vernalis* Sarg., were immersed in Dursban® TI and 50WP (chlorpyrifos), Oftanol® 2F (isofenphos), Turcam® 76WP (bendiocarb), Scimitar® 10CS (lambda-cyhalothrin), and Dylox® 80W (trichlorfon) for 30 seconds. They found Dursban® (both formulations) and Oftanol® to be the most efficacious in controlling white grubs, largely European chafer, *Rhizotrogus majalis* (Razoumowsky).

Three studies were conducted in 1996, 1998 and 1999 at a commercial nursery in Lake County, OH. The objective of the 1996 study was to evaluate the effects of size of root ball, soil type, insecticide rate and formulation and duration of immersion on the efficacy of insecticide dips. The objective of the 1998 study was to specifically evaluate the recommended rate and a reduced rate of Dursban® on three root ball sizes. The objective of the 1999 study was to further evaluate reduced rates of Dursban® for efficacy against Japanese beetle grubs and potential phytotoxicity of host trees.

Materials and Methods

1996 Dip test. In March, trees were mechanically dug at three Ohio nurseries suspected of harboring Japanese beetle. Trees were transferred to a commercial nursery equipped for dipping root balls. Star magnolia, *Magnolia stellata* (Siebold and Zucc.) Maxim, green ash, *Fraxinus pennsylvanica* Marsh, small-leafed linden, *Tilia cordata* Mill., and red maple, *Acer rubrum* L., with 61.0 or 81.3 cm (24 or 32 in) root balls, dug from two nurseries with sandy, well-drained soil, were immersed for 1, 2 or 5 min in water (control), Dursban® 4E or Dursban® 50WP at 0.906 kg ai/378.5 liter (2.0 lbs ai/100 gal), or Oftanol® 2F at 90.7 or 181.4 g ai/378.5 liter (0.2 or 0.4 lbs ai/100 gal). Sargent crabapple, *Malus sargentii*, with 61.0 cm (24 in) root balls dug from a third nursery with poorly drained, clay soil were subsequently dipped for 2 and 5 mins in Dursban® 4E at 0.906 kg ai/378.5 liter (2.0 lbs ai/100 gal) or Oftanol® 2F at 90.7 g ai/378.5 liter (0.2 lbs ai/100 gal). Control trees were dipped in water for 5 min.

There were 4 single-tree replications for each treatment. Groups of 4 trees were placed on a wood pallet and lowered into a surface-level tank filled with the insecticide solution. At the time of dipping, root ball temperatures ranged from 0 to 6.1C (32 to 43F) and the ambient temperature remained below 10.0C (50F) throughout the day. One week after dipping, all trees were transferred to the Ohio Agricultural Research and Development Center in Wooster, OH, where the test was evaluated five weeks after dipping. All soil from each root ball was thoroughly examined, and the species of each live grub was determined. Transformed data (sqrt X + 0.5) were subjected to analysis of variance. Means were separated with Tukey's multiple range test ($P < 0.05$) (1).

1998 Dip test. Norway maple, *Acer platanoides* L., pin oak, *Quercus palustris* Muenchh., Tatarian maple, *Acer tataricum* L. and Washington hawthorn, *Crataegus phaenopyrum* Medic, growing in Wooster silt loam soil were artificially infested with Japanese beetles in early July. Approximately 100 adult beetles (50:50 male:female) were confined in one small plastic cage on the ground at the base of each tree where they were allowed to oviposit. Each cage was made from a 5.8 liter Rubbermaid® storage box 32 × 17 × 11 cm (12.6 × 6.7 × 4.3 in). Two holes 11 cm diameter (4.3 in) cut in the bottom were covered with a small mesh screen that would not allow the beetles to pass through. A third hole 3.5 cm (1.4 in) diameter cut in the corner was used to introduce beetles into the box. The hole was sealed with a cork stopper after introducing beetles. The boxes were partially buried with the bottom of the box facing up. Beetles were added to the cages weekly throughout the month of July. In early September, five second and third instar Japanese beetles were individually buried 5.1 cm (2 in) deep around the base of each tree. In mid-September, pin oak, Tatarian maple and hawthorn were mechanically dug with 30.5 cm (12 in) root balls, while Norway maple were dug with 45.7 and 61.0 cm (18 and 24 in) root balls. The B&B stock was then transferred to the commercial nursery for dipping.

Root balls were immersed in water (control) or one of two rates of Dursban® 4E [0.453 and 0.906 kg ai/378.5 liter (1 and 2 lbs ai/100 gal) for 2 min. There were 15 single-tree replications per treatment. Groups of 5 to 15 trees were placed on a wood pallet and lowered into a surface-level tank filled with the insecticide solution. There were 15 each of pin oak, Tatarian maple and hawthorn with 30.5 cm (12 in) root balls. The trees were randomly distributed among the three treatments. All the 45.7 and 61.0 cm (18 and 24 in) root balls were Norway maple. Soil temperature of the root balls averaged 7.2C (45F) at the time of dipping. Approximately 60 days after dipping, 10 replications of each treatment were evaluated for the presence of white grubs. Data were analyzed as described previously.

One week after dipping, 5 trees of each treatment were transferred to the Ohio Agricultural Research and Development Center in Wooster, OH, and planted for phytotoxicity evaluation. Qualitative phytotoxicity observations were made 7–12 months after dipping. All the trees planted with 45.7 and 61.0 cm (18 and 24 in) root balls were Norway maple. The trees planted with 30.5 cm (12 in) root balls were made up of hawthorn, Tatarian maple and pin oak as follows: Con-

trol trees consisted of 5 Tatarian maples, the 0.453 kg ai/378.5 liter (1 lb ai/100 gal) treatment consisted of 3 hawthorns, 1 Tatarian maple and 1 pin oak, and the 0.906 kg ai/378.5 liter (2 lb ai/100 gal) treatment consisted of 5 pin oak.

1999 Dip test. Fifty honeylocust (*Gleditsia triacanthos* L.) growing in Wooster silt loam soil were artificially infested with Japanese beetle as described previously. Adult beetles were placed in the cages four times throughout the summer months. In early September, third instar Japanese beetles were individually buried 5.1 cm (2 in) deep around the base of each tree. In late September each tree was mechanically dug with 61.0 cm (24 in) root ball. The B&B stock was then transferred to a commercial nursery for dipping.

Treatments included a control (water), and 0.453, 0.226, 0.113 kg ai/378.5 liter (1, 0.5, 0.25 lb ai/100 gal) Dursban® 4E. All root balls were dipped for 2 min. Each treatment was replicated 10 times. Groups of 5 trees were secured to a wood pallet and lowered into a surface-level tank filled with the insecticide solution. Soil temperature of the root balls averaged 1.7C (35F) at the time of dipping. Approximately 60 days after dipping, the root balls were evaluated for the presence of white grubs. Data were analyzed as described previously.

Results and Discussion

In the 1996 study, most of the live grubs recovered from root balls of trees from the two northern nurseries were oriental beetle, *Exomala orientalis*, and most of the live grubs recovered from root balls of trees from the southern nursery were Japanese beetle. Overall, there were no differences among the insecticide treatments which reduced the number of live grubs 85–100% compared to the controls (Table 1). The grubs in these root balls included oriental beetle, European chafer, Asiatic garden beetle, *Maladera castanea* (Arrow), Japanese beetle and northern masked chafer, *Cyclocephala borealis* Arrow. Time of immersion, root ball size, and soil type had no effect on the efficacy of the dip treatments. Dead and decaying grubs were found in all insecticide-treated root balls. All the insecticide treatments of 61 cm (24 in) root balls from northern Ohio had significantly fewer grubs compared with the 1-min control ($F = 3.615$; df = 14, 45; $P < 0.001$). The number of grubs in all of the insecticide treatments of the 61cm (24 in) root balls was also less than the 2 and 5-min control treatments, however this reduction in grubs was not significant. The 81.3 cm (32 in) root balls from northern Ohio averaged 0 to 0.5 live grubs in the treated root balls and 21.5 in the control. This reduction of grubs was not significant ($F = 2.458$; df = 4, 15; $P = 0.091$).

Table 1. Effect of insecticide dip treatments in 1996 on the number of live scarab grubs recovered from root balls of B&B nursery stock.

Soil type	Root ball size cm (in)	Treatment	Rate kg (lb) ai/100 gal	Dip time (mins)	Number of live grubs/root ball (Mean ± SE)[z]
Sand	61.0 (24)	Control	(water only)	1	33.7 (24.4) a
		Control	(water only)	2	4.7 (1.2) ab
		Control	(water only)	5	8.5 (3.2) ab
		Dursban 50WP	0.906 (2.0)	1	1.0 (0.6) b
		Dursban 50WP	0.906 (2.0)	2	0 b
		Dursban 50WP	0.906 (2.0)	5	0.3 (0.2) b
		Dursban 4E	0.906 (2.0)	1	0 b
		Dursban 4E	0.906 (2.0)	2	0 b
		Dursban 4E	0.906 (2.0)	5	0 b
		Oftanol 2F	0.091 (0.2)	1	1.0 (0.4) b
		Oftanol 2F	0.091 (0.2)	2	0 b
		Oftanol 2F	0.091 (0.2)	5	0 b
		Oftanol 2F	0.181 (0.4)	1	1.3 (0.6) b
		Oftanol 2F	0.181 (0.4)	2	0.8 (0.5) b
		Oftanol 2F	0.181 (0.4)	5	0 b
Sand	81.3 (32)	Control	(water only)	5	21.5 (17.3) a
		Dursban 4E	0.906 (2.0)	2	0.5 (0.5) a
		Dursban 4E	0.906 (2.0)	5	0 a
		Oftanol 2F	0.091 (0.2)	2	0.5 (0.3) a
		Oftanol 2F	0.091 (0.2)	5	0.5 (0.3) a
Clay	61.0 (24)	Control	(water only)	5	2.5 (1.3) a
		Dursban 4E	0.906 (2.0)	2	0 b
		Dursban 4E	0.906 (2.0)	5	0 b
		Oftanol 2F	0.091 (0.2)	2	0 b
		Oftanol 2F	0.091 (0.2)	5	0 b

[z]Means followed by different letters are significantly different (Tukey's multiple range test, $P < 0.05$, based on [sqrt(x + 0.5)] transformed data, n = 4)

Table 2. Effect of 2-min insecticide dip treatments in 1998 on the number of live Japanese beetle grubs recovered from root balls of B&B nursery stock.

Treatment	Rate kg (lb) ai/100 gal	Root ball size cm (in)	Mean no. live JB grubs[z]
Control	(water only)	30.5 (12)	1.7ab
	(water only)	45.7 (18)	3.1a
	(water only)	61.0 (24)	4.9a
Dursban 4E	0.453 (1)	30.5 (12)	0.0b
		45.7 (18)	0.0b
		61.0 (24)	0.0b
Dursban 4E	0.906 (2)	30.5 (12)	0.0b
		45.7 (18)	0.0b
		61.0 (24)	0.0b

[z]Means followed by different letters are significantly different (Tukey's multiple range test, $P < 0.05$, based on [sqrt(x + 0.5)] transformed data, n = 10).

No live grubs were found in the 61.0 cm (24 in) treated root balls from southern Ohio, which was significantly different from the control ($F = 2.578$; df = 4, 15; $P = 0.005$). Phytotoxicity was not evaluated.

In the 1998 study, no live grubs were found in any of the insecticide-treated root balls, while an average of 1.7, 3.1 and 4.9 live Japanese beetle grubs were recovered from 30.5, 45.7, and 61.0 cm (12, 18 and 24 in) root balls dipped in water, respectively ($F = 12.2$; df = 8, 81; $P < 0.001$) (Table 2). A mean of 0.6 dead grubs was found in the 45.7 cm (18 in) root balls dipped in the 0.453 kg ai/378.5 liter (1 lb ai/100 gal) treatment, and 0.2 dead grubs were found in the 61.0 cm (24 in) root balls dipped in the 0.906 kg ai/378.5 liter (2 lb ai/100 gal) treatment. Dead grubs were not found in any other treatment.

The 61.0 cm (24 in) root balls dipped in water (control treatment) and dipped in the low rate of Dursban® showed no signs of phytotoxicity 12 months after dipping. Three of the five 61.0 cm (24 in) root balls dipped in the high rate of Dursban® [0.906 kg ai/378.5 liter (2 lbs ai/100 gal)] had smaller leaves compared with the control trees. One of these trees died 12 months after dipping. One tree from the 45.7 cm (18 in) root ball control treatment died 12 months after dipping, but no other signs of phytotoxicity were noted among the other trees in the control treatment. Three of five trees with 45.7 cm (18 in) root balls immersed in the 0.453 kg (1 lb) rate of Dursban® and all five root balls immersed in the 0.906 kg (2 lb) rate had smaller leaves when compared with the control trees. One tree dipped in the low rate and 2 trees dipped in the high rate died 12 months after dipping. None of the control trees with 30.5 cm (12 in) root balls showed signs of phytotoxicity. The trees with 30.5 cm (12 in) root balls (3 hawthorn, 1 Tatarian maple, and 6 pin oak) dipped in either rate of Dursban® were greatly affected. Four of the five trees immersed in the 0.453 kg (1 lb) rate and all 5 of the trees dipped in the 0.906 kg (2 lb) rate were dead 8 to 11 months after dipping. Of the 9 trees with 30.5 cm (12 in) root balls that died, 6 were pin oak, 2 were hawthorn and 1 was Tatarian maple. Although specific conclusions cannot be made about phytotoxicity of the particular species within this test, because not all species were represented in the control, it is clear that there can be a problem with phytotoxicity and that tree species and root ball size may be factors.

In the 1999 study, no live grubs were found in any of the insecticide-treated root balls; an average of 7.7 grubs were found in the root balls dipped in water ($F = 5.929$; df = 3, 36; $P = 0.002$) (Table 3). An average of 0.1 dead grubs was found in each of the 0.113 and 0.226 kg (0.25 and 0.5 lb) treatments; no dead grubs were found in the 0.453 kg (1 lb) treatment. All live and dead grubs were identified as Japanese beetle.

Overall, immersing root balls of B&B nursery stock in Dursban® is an effective treatment for reducing or eliminating Japanese beetle grubs. In 1996, 85–100% control was achieved. In both 1998 and 1999, 100 percent control was achieved with all insecticide treatments. An important difference between the 1996 study and the studies in 1998 and 1999 was the amount of time between when the dip treatment was implemented and when it was evaluated. In 1996, the test was evaluated only 35 days after dipping, while in 1998 and 1999, the tests were evaluated 60 days after dipping. This may contribute to some of the variability in the range of control in 1996, as well as the 100% control achieved in 1998 and 1999. In 1996, there may not have been sufficient time between dipping and evaluation to reveal all the effects of the treatment.

Differential susceptibility among grub species may have also contributed to variation in control observed among the studies (6). In 1998 and 1999 only Japanese beetle grubs were recovered from root balls, while in 1996, a mix of white grub species was recovered with only a small percentage Japanese beetle. Time of year may have also influenced this variability in that the tests in 1998 and 1999 were conducted in the fall which would target third instars that had not overwintered. The test in 1996 was conducted in March which targeted older third instars that had overwintered and may have been more difficult to kill.

Although immersing the root balls of B&B nursery stock in Dursban® is an effective treatment for reducing or eliminating Japanese beetle grubs, phytotoxicity may occur. Tree species and root ball size may influence tree survival after dipping. Apparently, not all species will be suitable for dip treatments. More studies are needed to determine which tree species are sensitive and when trees are most vulnerable to phytotoxicity from this treatment. A reduced rate of Dursban® may minimize phytotoxicity problems. One-eighth the recommended rate used in 1999 provided 100% control.

Immersing the root balls of B&B nursery stock in insecticide solutions can be difficult, messy and potentially hazardous. However, these and other studies have shown it to substantially reduce or eliminate Japanese beetle grubs. This method also permits treatment of nursery stock shortly be-

Table 3. Effect of rate of Dursban 4E dip treatments for 2 min on the number of live Japanese beetle grubs recovered from 61.0 cm (24 in) root balls of B&B nursery stock.

Treatment	Rate kg (lb) ai/100 gal	Mean no. live JB per root ball[z]
Control (water)	—	7.7a
Dursban 4E	0.113 (0.25)	0.0b
Dursban 4E	0.226 (0.5)	0.0b
Dursban 4E	0.453 (1.0)	0.0b

[z]Means followed by different letters are significantly different (Tukey's multiple range test, $P < 0.05$, based on [sqrt(x + 0.5)] transformed data, n = 10).

fore shipping (3 days) as an alternative to soil insecticide applications in late spring to mid-summer that target young, actively feeding Japanese beetle grubs. These types of studies are supported by the USDA, The National Plant Board, and the regulated industry, and will provide a scientific basis for recommendation of the most reasonable and efficacious treatments to reduce the dispersal of Japanese beetle.

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