Effect of seed size, colour, number of seeds per hill and depth of planting on sorghum seed survival and stand establishment: relationship to phytophagous insects

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Sorghum, *Sorghum bicolor* (L.) Moench, seeds were planted in two different cropping systems, 'casado' (sorghum and maize in the same hill) and 'golpe alterno' (sorghum and maize in alternating hills) in one test; and in monocolture in a second test to determine the effects of number of sorghum seeds hill^{-1} and depth of planting, and seed size and colour, respectively, on seed survival and stand establishment in an area in Central Honduras infested with seed and seedling insect pests. Seed size and colour did not influence the amount of damage to seeds and seedlings by insect pests. More seeds were destroyed or removed where seeds were planted 7.5 cm deep than at 2.5 cm. Seedling emergence was higher when seeds were planted 2.5 cm deep than at 7.5 cm. Seeds were damaged less by insects, and seedling emergence was higher in the alternate hill system than in the same hill system. The loss of seeds and reduction in plant stand was due mainly to ants (*Solenopsis geminata* (F.)), although white grubs (*Phyllophaga* sp.) and fall armyworms (*Spodoptera frugiperda* (J. E. Smith)) contributed to stand destruction.

Keywords: Sorghum; Seed size; Seed colour; Seeds hill^{-1}; Planting depth; Seed survival; Stand establishment; Phytophagous insects

The establishment of a good crop stand is critical for resource-poor subsistence farmers in developing countries. Insects and low soil moisture are two important causes of poor seed germination and seedling damage. Where moisture is not available near the soil surface, seeds must be planted deep. Petrini *et al.* (1984a) reported that sweet sorghum seed had highest seedling emergence and stand density when seeds were planted at a depth of 5.5 cm compared with 1.5, 3.5 or 7.5 cm. Petrini *et al.* (1984b) also found that germination rate and plant dry weight increased with increased planting depth although final fresh yield was not affected. Black (1956) reported that planting depths of 1.3–5.8 cm resulted in uniform germination of subterranean clover, *Trifolium subterraneum* L., whereas alfalfa seedling emergence was reported to decrease as planting depth increased (Beveridge and Wilsie, 1959). Tischler and Voigt (1983) reported that deep planting of seeds of several grass species resulted in reduced seedling vigor and subsequent plant performance was adversely affected.

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Critical depth of planting is determined by seed size and type. Most crops have an optimum depth of planting (Black, *ibid.*). The larger the seed size the greater the capacity of its seedlings to emerge from deeper locations in the soil (Van der Valk, 1974). Little information is available concerning the effects of depth of planting in relation to seed predation or seedling damage by insects. Abramsky (1983) reported that ants, *Messor arenarius*, were not effective in finding seeds below the soil surface.

The optimum number of seeds to be planted is also important in crop production systems. This is especially important to subsistence farmers who have a limited amount of seed for planting. DeWalt and DeWalt (1982) reported that farmers in southern Honduras planted 12–15 sorghum seeds hill^{-1}; it is estimated that 3.6–7.7 kg of sorghum seeds were used ha^{-1}. Replanting may not be possible due to the lack of seed, cost of additional seed or lack of soil moisture for a later second planting. Therefore, the establishment of a good plant stand after the initial planting is critical in most subsistence farming operations. Trabanino *et al.* (1987) reported that sorghum seed treated with Promet® (furathiocarb)

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and planted 7.5 cm deep in a field where phytophagous insects were abundant resulted in only 58% stand establishment; untreated seed gave only 3% stand establishment.

Sorghum seed colour ranges from dark brown to red to white, depending on variety. Brown seeds contain high levels of phenolic compounds, such as tannins, which create an offensive taste to birds (Tipton *et al.*, 1970; McMillan *et al.*, 1972, Price *et al.*, 1979) and insects (Woodhead *et al.*, 1980). Brown and white seed types were compared to determine the effects of seed size and colour. Also studied were the effects of number of seeds and depth of planting on seed survival and stand establishment in an area infested with seed and foliage-feeding insects.

Materials and methods

Two experiments were conducted in 1986 at the Panamerican Agricultural School, located 35 km east-southeast of Tegucigalpa, Honduras.

Study 1

This study was conducted 8–28 June 1986 using seeds of sorghum, *Sorghum bicolor* (L.) Moench (of the tropical variety 'San Bernardo III') and maize, *Zea mays* L. Seeds were planted at two different depths (2.5 cm and 7.5 cm) in hills 50 cm apart and in rows 80 cm wide, using a digging stick ('chuzo') that penetrated the soil only 2.5 or 7.5 cm deep. Sorghum seeds were planted in moist soil at three rates, 5, 10 or 20 seeds $hill^{-1}$, and in two different cropping systems, (a) 'casado' (sorghum and maize in the same hill) and (b) 'golpe alterno' (sorghum and maize in alternating hills). These two systems are commonly used by subsistence farmers in Honduras. The maize seeds were used at a rate of three seeds $hill^{-1}$ in all treatment plots. Six treatments were evaluated in each of the two cropping systems: five sorghum seeds planted 2.5 cm deep, 10 seeds at 2.5 cm, 20 seeds at 2.5 cm, 5 seeds at 7.5 cm, 10 seeds at 7.5 cm and 20 seeds at 7.5 cm.

The test was established as a 3 × 2 × 2 factorial with treatments arranged in a randomized complete block design with four replications. Treatment plots in the same hill system were 5 × 3.2 m, and 10 × 3.2 m in the alternate hill system to equalize plant number $treatment^{-1}$. Each treatment plot consisted of four rows, 5 or 10 m long; the two middle rows were utilized for sampling. Pre-planting and 10-day-post planting soil samples (20 × 20 cm) were taken at 10 random sites in each field, using a hoe 20 cm wide to a depth of 10 cm; the samples were searched visually to determine the complex of insect pests in the study area. Post-planting plant and insect data were recorded on days 5, 10, 15 and 20 and included observations on the number of missing seeds (counts of seeds in hills), number of dead seeds, number of seedlings emerged, plant stand, number of dead plants, plant height, number of leaves $plant^{-1}$ and number of insects in each hill. The data for each date were analyzed using arcsin transformations of the percentages for analysis of variance; means were separated by Student-Newman-Keuls' test (Steele and Torrie, 1960).

Study 2

This study was conducted 28 June–17 July 1986. A brown seed sorghum hybrid, [(SC326-6 × SC103-12) Liberal]-40, and a white seed variety, San Bernardo III TP Zs B1b2, were used. Small and large seeds of each sorghum were evaluated in monoculture, using the mean weight of 100 seeds as an indicator of size. Treatments included small white seeds, 2.2 g; large white seeds, 3.6 g; small brown seeds, 1.5 g; and large brown seeds 2.6 g. Percentage seed germination was 92 and 85 for the white and brown seeds, respectively.

Treatment plots were arranged in a randomized complete block design with four replications. Each treatment plot consisted of four rows, 5 m long, the middle two rows being utilized for sampling. Sorghum seeds were planted 7.5 cm deep, using a digging stick, in hills 50 cm apart and 80 cm between rows. This planting depth is commonly used by subsistence farmers in southern Honduras. Each hill contained 10 sorghum seeds. Observations were made on days 5, 10, 15 and 20 on number of missing seeds, number of dead seeds, number of seedlings emerged, number of dead plants and number of insects in each hill. The data were analyzed as in Study 1.

Results and discussion

Pre- and post-planting soil samples indicated that ants, *Solenopsis geminata* (F.), white grubs, *Phyllophaga* sp., and fall armyworm, *Spodoptera frugiperda* (J. E. Smith) were the most common pest insects in the field plots. A chi-square test suggested that these insects were uniformly distributed in the fields. Ants were frequently observed removing seeds from planting sites and the populations appeared to be higher than usually encountered in intercropped sorghum/corn production fields in southern Honduras.

Study 1

Differences in treatment main effects were observed, except in number of seed $hill^{-1}$. However, only the data for those variables on specific sample dates with significant F values ($P = 0.10$) are reported (Table 1). Sorghum seedling emergence was higher on days 5 and 10 after planting when seeds were planted 2.5 cm deep than when the seeds were planted 7.5 cm deep. The percentage of missing sorghum seeds on day 10 after planting was the inverse of seedling emergence 5 and 10 days after planting. More sorghum seeds were destroyed or removed where seeds were planted 7.5 cm deep than at 2.5 cm. This loss of seeds was observed to be mainly due to ants, which were capable of locating the seeds in the deep planting sites. More critical observations are needed to determine why more seeds were removed from deep planting sites than shallow sites. The moisture was sufficient in both locations to stimulate equally rapid germination, but the deep-planted seeds would be expected to have delayed emergence, allowing more time for insects and other pests to destroy or remove the seeds prior to seedling emergence. Tischler and Voigt (1983) explained that the failure of seeds to emerge when planted at excessive depths is due to insufficient

Table 1 Effect of planting method on sorghum stand and establishment. El Zamorano, Honduras, 1986

Method	Percentage (± SEM) emerged plants on days after planting 5	10	Percentage (± SEM) missing seeds on days after planting 5	10	Mean (± SEM) plant height[a] (cm) on day 15 after planting
Planting depth (cm)					
2.5	43.5 ± 9.2 a[b]	42.1 ± 14.6 a	67.0 ± 10.0 a	58.4 ± 5.9 a	4.7 ± 0.2 a
7.5	28.8 ± 4.5 b	27.0 ± 1.8 b	49.2 ± 5.7 b	71.1 ± 1.9 b	4.0 ± 0.4 b
System[c]					
Same hill	29.7 ± 7.5 a	—	—	—	—
Alternate hill	42.5 ± 8.1 b	—	—	—	—

[a]Height measured to base of whorl
[b]Means in a column within method not followed by the same letter are different at $P = < 0.10$ level by Student-Newman-Keuls' test. The $P = 0.10$ level is used to make comparisons more sensitive
[c]Same hill, corn and sorghum in same hill; alternate hill, corn and sorghum in alternating hills

Table 2 Effects of number of seed per hill, depth of planting and planting system on sorghum stand and establishment. El Zamorano, Honduras, 1986

Interaction[a]	Missing seeds (%) day 10	Dead seeds (%) day 5	Cut plants (%) day 15	Cut plants (%) day 20	Plant stand (%) day 15	Plant stand (%) day 20	Mean height day 10	Mean height day 15
System × depth	N	S	S	N	N	N	N	S
System × seeds	N	N	N	N	N	S	S	S
Depth × seeds	S	N	N	S	S	N	N	S
System × depth × seeds	N	N	S	N	S	N	N	N

[a]Interactions in three way factorial analysis of variance were significant (S) or not significant (N), $P < 0.10$. The $P = 0.10$ level is used to make comparisons more sensitive

seedling energy to push through the soil, particularly when unfavorable conditions for germination and seedling emergence exist, but deep planting may improve seedling emergence and establishment because of the presence of adequate moisture where the seeds are planted. Plants developing from seeds planted deeply may not only have delayed emergence, but young plants will also show initial slow development. In this study, plants developing from seeds planted 2.5 cm deep were taller on day 15 after planting than plants developing from seeds planted 7.5 cm deep (Table 1).

Sorghum seedling emergence was significantly higher on day 5 in the alternate hill system than in the same hill system, and the opposite was observed for missing seeds in these plantings at this time (Table 1). The maize, planted in the same hill with sorghum seeds, may be a factor in attracting phytophagous pests and thus more seeds are destroyed or removed from the same hill system than from the alternate hill system. Another factor may have been the increased plant density in same hill plots which allowed ants to find and forage more easily than in the alternate hill plots. Additional research is needed to elucidate the relationship of maize seeds with sorghum seeds in considering pest damage to the seeds in different planting systems.

Interactions were observed between system and depth of planting (dead seed on day 5, cut plants and height on day 15), system and number of seeds (height on day 5, 10 and 15, stand on day 20), depth of planting and number of seeds (missing seeds on day 10, stand and height on day 15, cut plants on day 20), and system × depth of planting × number of seeds hill^{-1} (stand and cut plants on day 15) (Table 2). Variables with treatment interactions showed differences among the treatments in eight of 12 analyses. Data on missing seed and plant stand and height showing interaction of planting depth and number of seeds hill^{-1} present an indication of the effects of these treatments (Table 3). Although missing seed was involved in an interaction, differences among treatments on day 10 after planting were not observed, indicating that planting depth and number of seeds hill^{-1} did not affect insect predation. Thus, no clear definition of the independent effects of depth of planting and number of seeds hill^{-1} on insect damage to sorghum seeds and emerging seedlings can be identified (Table 3).

Plant stand on day 15 after planting was not affected by number of seeds planted at the 2.5 cm depth, but was affected at the 7.5 cm depth. Seedlings emerged from the 2.5 cm depth to establish a stand before seedlings emerged from 7.5 cm depth. The larger number of plants in the 20 seed hill^{-1} treatment plots compared with the lower number of plants when fewer seeds were planted at 7.5 cm deep may be related to the greater success of plant emergence with increased number of seeds hill^{-1}. The seedlings in hills with 20 seeds were able to push through the soil with less difficulty and more quickly than the plants in hills with fewer seeds.

Plant height may be used as an indicator for evaluating the effect of insect damage to plants as discussed previously, but no clear trend in plant height was observed over all sample dates when comparing the effects of number of seeds hill^{-1} and planting depth in this study (Table 3). Nevertheless, an unexplained significant difference in plant height was observed between treatments on day 15 after

Table 3 Effects of depth of planting and number of seeds per hill on sorghum stand. El Zamorano, Honduras, 1986

Planting depth (cm)	Number of seeds hill^{-1}	Percentage (± SEM) missing seeds on day 10 after planting	Percentage (± SEM) plant stand on day 15 after planting	Mean (± SEM) plant height (cm)[a] on day 15 after planting
2.5	5	56.3 ± 7.5 a[b]	47.5 ± 8.5 a	4.9 ± 0.2 a
2.5	10	50.9 ± 8.9 a	23.8 ± 7.3 ab	4.8 ± 0.1 a
2.5	20	68.1 ± 10.5 a	29.5 ± 7.3 ab	4.4 ± 0.7 ab
7.5	5	73.1 ± 5.6 a	8.8 ± 2.1 b	3.0 ± 0.9 b
7.5	10	80.3 ± 5.7 a	25.6 ± 7.0 ab	3.9 ± 0.8 ab
7.5	20	59.8 ± 8.7 a	45.6 ± 7.1 a	5.0 ± 0.3 a

[a]Height measured to base of whorl
[b]Means in a column not followed by the same letter are different at $P < 0.10$ level by Student-Newman-Keuls' test. The $P < 0.10$ level is used to make comparisons more sensitive

Table 4 Effects of sorghum seed size and seed colour on missing seeds, seedling emergence and plant stand establishment. El Zamorano, Honduras, 1986

Seed[a]		Percentage (± SEM) missing seeds on day 5 after planting	Percentage (± SEM) emerged plants on days after planting		Percentage (± SEM) live plants on days after planting		Percentage (± SEM) cut plants on day 20 after planting
Size	Colour		5	10	15	20	
Small	Brown	71.3 ± 8.9 a[b]	24.4 ± 10.8 a	10.6 ± 9.0 a	9.3 ± 7.1 a	5.0 ± 5.0 a	0.00 ± 0.0 a
Small	White	60.0 ± 15.5 a	38.1 ± 15.7 a	9.4 ± 9.4 a	8.7 ± 5.1 a	4.4 ± 2.7 a	4.40 ± 2.2 b
Large	Brown	72.5 ± 12.1 a	33.1 ± 15.3 a	10.0 ± 6.1 a	11.3 ± 8.3 a	2.5 ± 2.5 a	0.60 ± 0.6 b
Large	White	80.0 ± 7.1 a	18.8 ± 7.3 a	13.8 ± 5.2 a	11.3 ± 1.3 a	8.8 ± 6.5 a	0.60 ± 0.6 b

[a]Brown seed, [(SC326-6XSC103-12)Liberall-40; white seed, San Bernardo III TP Zs B1b2
[b]Means in a column not followed by the same letter are different at $P = < 0.05$ level by Student-Newman-Keuls' test

planting. Number of seeds planted 2.5 cm deep had no effect on plant height on day 15 after planting; this may be expected, since seedlings emerged at about the same time and did not experience stress during this period of early plant growth. In general, plants developing from seeds planted 7.5 cm deep were shorter on day 15 after planting than plants developing from seeds planted 2.5 cm deep. We might expect shorter plants when seedling emergence is delayed and the plants are subjected to a longer period for damage by pests, as well as when seedlings are in competition for nutrients; this is probably what occurred when seeds were planted deep (except for unexplained data for 20 seeds at 7.5 cm deep).

Study 2

Comparisons of seed size and seed color in relation to damage by soil-inhabiting insects showed that the proportion of missing seeds (range 60–80%) from treatment plots was not different on day 5 after planting (Table 4). No preference for size or colour was exhibited by the insects encountered in the study area, although the observed values in percentages of missing seeds were large white 80, large brown 73, small brown 71 and small white 60. Additionally, there were no differences in the number of dead seeds recovered from treatment plots.

Very little information is available in the literature relating seed size and colour to insect damage. Most studies have been conducted with stored grain pests. Nelson and Johnson (1983) showed that bruchid beetles preferred large seeds to medium or small seeds. As large seeds are recognized as having more total nutrients than small seeds, we might expect them to be more acceptable to certain insects, mainly because of their reproductive relationship with the preferred larger seeds that provide more food resources to insure the completion of development. However, some small insects, e.g. ants, that feed directly on the seed when the seed is too large to be carried away (as was the case with maize) feed on the seed at the site where it was planted. The insects encountered in this study were not handicapped in their foraging and feeding by the general small size of sorghum seeds.

Colour also did not have a significant effect on seed damage. Rogler (1985) reported that tannin toxicity was not the same every year and that it is possible that the chemistry of tannin may be altered by growing conditions. Further studies are needed to identify the role of sorghum seed colour and, specifically, the influence of tannins on insect damage to sorghum seeds. Particular attention should be given to the reaction of ants to seeds with different well-defined chemical components, especially tannin.

Seedling emergence was low (Table 4) and was a reflection of the high percentage of seeds removed from the hills by ants and other insects. The four treatments on days 5 and 10 after planting did not differ significantly. The reductions (5 to 23%) in plant stands in treatment plots from day 5 to day 10 after planting, in the absence of plant diseases, clearly suggest the harmful impact of insects on the establishment of sorghum plants in the field. Plant stands in plots on days 15 and 20 after planting were consistently similar in number of live plants on each day and at levels less than 12% of the number of seed planted (Table 4). Fall armyworms, acting as cutworms, were responsible for some reduction in plant stand. This reduction was observed mainly in plant developed from small white seeds and plants developing from large brown and large white seeds, which were significantly more damaged in relation to plants developing from small brown seeds (Table 4). Studies reported by York (1981) indicated that on the basis of equal weight of seeds, the relative amount of tannin present is many-fold greater in small seeds than in larger seeds. This higher amount of tannins in small seeds may be responsible for the low numbers of fall armyworms from these plots. Although white grubs were abundant, damage by these insects could not be accurately measured.

The small number of plants surviving at 20 days after planting of untreated seed is sufficient evidence to recommend the need for insect control measures, beginning with protection of the sorghum seed. Trabanino *et al.* (1987) reported that chemical (insecticides or kerosene) seed treatments provided some protection to sorghum seed during germination and stand establishment. Some chemicals should not be used or be used with caution due to phytotoxicity. They suggested that excess seeds should be planted, even when chemical seed treatments are used, to compensate for the loss of sorghum seeds and reduction in plant stand from insect pests and other causes.

The present studies confirm the effects of insects in reducing sorghum plant stand when sorghum is planted alone or intercropped with maize. The results suggest that size and colour of sorghum seeds did not influence the amount of damage to seeds and seedlings by insect pests in the study area in Honduras. The number of seeds planted and depth of planting influenced seedling stand establishment; plant stand was inversely correlated with missing seed. Seeds planted 2.5 cm deep survived insect and other pest damage and established a better crop stand compared with seeds planted 7.5 cm deep. Also, sorghum and maize planted in 'golpe alterno' appeared to be damaged less by insects than 'casado' planting. Additional studies are needed to obtain a better understanding of sorghum seed size and color, and number of seeds planted and depth of planting in relation to seed loss and reduction in plant stand.

Although studies should be conducted with sorghum planted in conventional planting systems in areas of Honduras, and in other locations where pest problems differ, the loss of sorghum seeds and plant stand in intercropping systems is critical to the economy of subsistence farmers. The determination of pest constraints to sorghum production during seed germination and seedling emergence and plant stand establishment is critical to understanding pest problems and developing recommendations for control of insects and other pests.

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Time of artificial insemination and pregnancy rates in Boran (*Bos indicus*) cattle

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The fertility of Boran cattle was determined following artificial insemination (AI) at different times from the onset of induced oestrus. 46 animals were inseminated 6, 12 and 18 h after observed oestrus which was induced with a single injection of 25 mg prostaglandin F2 alfa (PGF2a: Lutalyse)[2]. Animals failing to respond were given a second injection after 12 days and inseminated at a fixed time, 72 h later. Ten animals served as untreated controls and were inseminated 12 h after observed natural oestrus during a 22-day breeding period. Overall oestral response to PGF2a treatment was 72%. Oestrus was observed an average of 76.9 ± 42.8 h after treatment. The pooled mean pregnancy rate (PR) was 37.5% and was significantly affected ($P < 0.05$) by AI time but not age group, bull or AI technician. PR was highest (56%) in animals inseminated 6 h after observed oestrus, similar to the 60% in the control animals. The lowest pregnancy rates (33%) were in animals inseminated either 12 or 18 h after induced oestrus. It was concluded that PGF2a treatment of Boran cows leads to earlier ovulation and fertilization, and treated animals need to be inseminated earlier than after natural oestrous.

Keywords: Zebu cattle; Artificial insemination; PGF2a; Synchronization

Bos indicus (Zebu) dominate the cattle population in the tropics (Landivar *et al.*, 1985). Individual animal productivity is, however, still low in many areas. Animals are therefore being crossed through natural breeding or artificial insemination with improved breeds from temperate areas. Although the use of AI in cattle is expanding worldwide (Hunter, 1985), fertility rates to AI in Zebu cattle have been rather disappointing. Oestrus in the Zebu tends to be shorter (Anderson, 1944; Plasse *et al.*, 1970; Zakari *et al.*, 1981) and is often subdued. Zebu cows may refrain from repeated mounting (Galina *et al.*, 1982; Landivar *et al.*, *ibid.*). Furthermore, when cows reared at pasture are confined for oestrus observation, their behaviour may be altered and heat may be silenced (Galina and Escobar, 1985).

Time of AI in relation to oestrus affects pregnancy rate in taurine cattle (Salisbury and Van Demark, 1961; Foote, 1979). Taurine cows may be inseminated 6–24 h after oestrus (Trimberger and Davis, 1943; MacMillan and Watson, 1975; Foote, 1975). Variation in fertility rates occurs due to differences in the interval between onset of oestrus and its detection (Foote, 1979); this arises from the frequency and the efficiency of the methods of oestrus detection (Esslemont and Bryant, 1976). The AM:PM rule is generally adopted in taurine cattle whereby animals observed in oestrus in the morning are inseminated in the afternoon and *vice versa*. There has been minimal success using this guideline with the Zebu. As a result, the fertility rates to AI are often low (Galina and Escobar, 1985), indicating the need for more research on their reproductive physiology. This study was undertaken to determine the conception rates of Boran cattle following AI at different times from the onset of induced oestrus compared with AI following natural oestrus.

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[2] Lutalyse is a dinoprost tromethamine (prostaglandin: PGF2a) product from Upjohn Co.

Materials and methods

74 animals were initially selected for this study. On rectal palpation, 17 were found to be either pregnant or had inactive ovaries (no *corpus luteum* or follicles) and were eliminated. The remaining 56 comprised 43 pluriparous cows and 13 heifers. The study was undertaken at the Abernossa cattle improvement and multiplication centre of the Ethiopian Ministry of Agriculture, 180 km south of Addis Ababa (Azage *et al.*, 1988). The body condition of all animals was scored on a scale of 1 to 9 (Nicholson and Butterworth, 1986). Animals were inseminated by two experienced resident technicians using frozen semen from five high-fertility Friesian bulls.

46 of the animals received a single injection of 25 mg PGF2a i.m. and were maintained under constant observation over the next six days for oestrus detection. Animals were inseminated 6 h after observed oestrus (Group 1, $n = 9$), 12 h after observed oestrus (Group 2, $n = 15$) or 18 h after observed oestrus (Group 3, $n = 9$). Ten animals were used as untreated controls and were inseminated 12 h after observed natural oestrus during a 22-day period (Buck *et al.*, 1980), following normal farm practice (Group 4). Thirteen treated animals that failed to show oestrus were given a second injection 12 days after the first injection and inseminated at a fixed time, 72 h after the injection (Group 5). Pregnancy rates were established by rectal palpation undertaken 45–60 days after service.

Data were analysed by analysis of variance using the General Linear Models Procedures at the ILCA

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