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Landscape-level changes in pest suppression as a result of large scale planting of *Bt* cotton

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Abstract

High adoption rates of genetically modified, insecticidal *Bt* cotton lead to regional changes in pest management practice with potential consequences for populations of the main lepidopteran pest, *Helicoverpa* spp., and natural enemies in *Bt* cotton and neighbouring crops. To test the hypothesis that *Bt* cotton functions as a sink and other crops as potential sources for *Helicoverpa* spp., we determined *Helicoverpa* spp. egg densities in 24 *Bt* cotton fields in three landscapes in the Darling Downs, QLD, Australia. Egg densities peaked in January 2010, when cotton started flowering, and decreased afterwards, except in one landscape, where a second peak was observed at the end of February. In this landscape, rainfall allowed planting of large areas of sorghum early in the season. Consequently, the late season peak is likely to be a result of third generation moths recruited from sorghum. In the two other landscapes, non-cotton crops were planted later in the season or not at all. While *H. punctigera* dominated the samples early in the season, *H. armigera* was most frequent in January, but *H. punctigera* populations increased again towards the end of the season. To test the hypothesis that natural enemies of *Helicoverpa* spp. eggs remain unaffected by *Bt* cotton, we followed parasitization rates of field collected and sentinel *Helicoverpa* spp. eggs and predation rates of sentinel eggs. Egg parasitoids were not found before January, but 40-80% of the field collected eggs were parasitized thereafter. Parasitoids were clearly dominated by Trichogrammatidae. From our samples, there was no indication that Bt cotton affected egg parasitoids or predators. The project is continuing in the field season 2010/11 with Australian funding from Cotton CRC and GRDC. Data will be collected in a similar way and more detailed analyses will be conducted with the complete data set.

Introduction

The worldwide area planted to genetically modified (GM) crops is steadily increasing, passing 134 million hectares in 2009 (James, 2009). While the majority of GM crops have been modified for herbicide tolerance, crops with resistance to certain insect pests are grown on 50.4 million hectares. More than half of this area (28.7 hectares) was planted to stacked traits with combined herbicide tolerance and insect resistance (James, 2009). Insecticide resistance is achieved by the expression of genes from the bacterium *Bacillus thuringiensis* (*Bt*) that encode insecticidal crystal (Cry) proteins. Cry proteins are known for their narrow spectrum of activity and have a long history of safe use as microbial *Bt* products (Glare & O' Callaghan, 2000). The major *Bt* crops in global commercial production are maize and cotton (James, 2009).

Cotton is grown in more than 75 countries and supplies almost 40% of total worldwide demand for fibre (Naranjo et al., 2008). Before *Bt* cotton was introduced, almost half of the insecticides used in agriculture were applied to cotton, with approximately 50% used against lepidopteran pests (Fitt, 2008). Current *Bt* cotton varieties produce Lepidoptera-specific Cry1 and Cry2 proteins targeting the most damaging pests, i.e. budworms and bollworms (Naranjo et al., 2008). Australia, the USA and Mexico were the first countries to permit commercial cultivation of *Bt* cotton in 1996 (Benedict & Ring, 2004; James, 2009). The largest proportional adoption is in Australia, where 94% of the cotton acreage was planted to *Bt* varieties in 2009 (James, 2009). There, the double gene cotton expressing Cry1Ac and Cry2Ab introduced in 2002, required 80-90% less applications of insecticides compared to conventionally managed fields (Brookes & Barfoot, 2008; Fitt, 2008).

Before GM crops can be grown commercially, regulation requires that ecological risks are assessed. With the introduction of *Bt* crops, particularly side effects on non-target species should be evaluated (Romeis et al., 2008). Studies conducted in the USA and Australia demonstrated that *Bt* cotton had only minor effects on natural enemy abundance and biological control function, while significant adverse effects were present after application of insecticides in conventional cotton (Naranjo, 2005a,b; Head et al., 2005; Whitehouse et al., 2005). The specificity of *Bt* crops preserves natural enemies that feed on a range of prey species that are not affected by the *Bt* protein. However, due to the highly efficient elimination of target species in *Bt* cotton and other *Bt* crops, predators and parasitoids that are specialized on target herbivores may suffer from the lack of prey and hosts (Naranjo, 2005a; Romeis et al., 2006).

Cotton as an annual crop is recolonized by herbivores and natural enemies every year from other crops and natural habitats. Recolonization can occur on a regional and national scale. For example, the *Helicoverpa* species causing problems in Australia, *H. punctigera* and *H. armigera,* show different recolonization patterns. The former species rarely over winters in significant numbers in the cropping areas of eastern Australia, recolonizes from inland Australia using suitable wind systems during the spring and usually declines after mid-January. In contrast, *H. armigera* diapauses in the eastern cropping region, recolonizes from habitats within the region and passes through 4-5 generations during the summer season from September to April (Schellhorn et al., 2008; Baker et al., 2008; Zalucki et al., 1986).

Natural enemies of *Helicoverpa* spp. include generalist predators (e.g., spiders, predatory bugs, predatory beetles, and lacewings) and parasitic wasps, which are often specialized on lepidopteran eggs, larvae and pupae (Fitt, 1994). For example, the egg parasitoids *Trichogramma* spp. and *Telenomus* spp. can be very effective with parasitisation rates exceeding 50% in cotton (Schellhorn et al., 2000).

Most research on arthropod populations and pest control function in *Bt* cotton and other *Bt* crops has been limited to field level, i.e. one or more *Bt* fields were compared to non-*Bt* fields. Arthropod populations, however, move on a landscape level and migrate into and out of fields.

High adoption rates of *Bt* cotton lead to changes in pest management practice (i.e. insecticide applications), which are likely to influence pest and natural enemy populations not only in the *Bt* cotton fields, but also in neighbouring, non-*Bt* cotton fields (Storer et al., 2008). Even though large scale planting of *Bt* cotton started a decade ago, consequences on a landscape-level have hardly been studied. Data collected in six Chinese provinces provide the first evidence that highly efficient control of *Helicoverpa armigera* in 3 million hectares of *Bt* cotton reduced the pest also in 22 million hectares of other crops grown in the same region (Wu et al., 2008). In the USA, cultivation of *Bt* maize for more than a decade resulted in region wide suppression of the European corn borer *Ostrinia nubilalis* Hübner, with benefits for non-*Bt* maize growers (Hutchison et al. 2010)

Research hypotheses

Our first hypothesis is that *Bt* cotton functions as a sink for *Helicoverpa* spp., because the moths do not discriminate between *Bt* and non-*Bt* cotton for egg laying and most larvae die quickly when feeding on *Bt* cotton. Recruitment for the population has to come from other crops that function as a source for *Helicoverpa* spp., e.g. sorghum, maize, or pigeon pea (Fig. 1). In consequence, recruitment should be lower in areas with a high percentage of *Bt* cotton compared to those with a high percentage of other *Helicoverpa* spp.-susceptible crops. This effect is expected to be more pronounced towards the end of the growing season, several generations after the initial colonization of the region. To test this hypothesis, we determined *Helicoverpa* spp. egg densities in *Bt* cotton fields and mapped the landscape around those fields. Correlations throughout the season show if and when the egg densities in fields surrounded by other cotton fields are lower than densities in fields surrounded by other *Helicoverpa* spp.-susceptible crops.

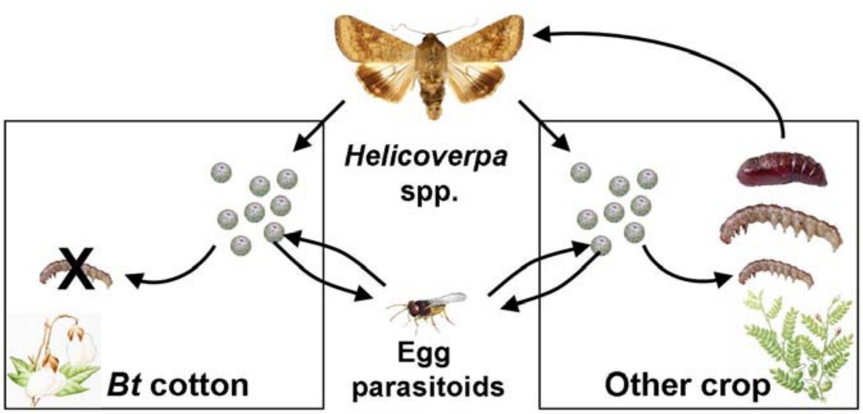

Fig.1: *Helicoverpa* spp. and egg parasitoid dynamics in *Bt* cotton and other crops

The second hypothesis is that natural enemies of *Helicoverpa* spp. eggs are unaffected by *Bt* cotton, because the eggs are not exposed to the Cry protein and are therefore not harmed (Fig. 1). In addition, the toxin acts specifically on lepidopteran species with no direct effects on natural enemies. Declining populations of *Helicoverpa* spp., however, may affect their natural enemies indirectly. In this project, we followed parasitization rates of *Helicoverpa* spp. eggs throughout the season. Parasitization rates are determined from both field collected eggs and sentinel eggs that are exposed to natural enemies for 2 days. The sentinel eggs will also allow drawing conclusions on predator activity.

Materials & Methods

Landscapes

Field work was conducted in the Darling Downs, Queensland, eastern Australia. This agricultural region is known for a diverse cropping pattern with the major summer crops being cotton, sorghum, mungbeans, maize, and sunflowers. Pigeon pea fields covering 5% of the cotton area on a farm were usually planted as part of the licence agreement for growing *Bt* cotton with the aim of delaying resistance development of *Helicoverpa* spp. Alternative refuges were 3 consecutive plantings of sorghum (15% of cotton area) or maize (20%), unsprayed, conventional cotton (10%), or sprayed, conventional cotton (100%) (Farell, 2008).

Three landscapes of ca. 10 km diameter were selected between the townships of Dalby and Millmeran, i.e. "Nandi", "Cecil Plains", and "Pampas" (Fig. 2).

Fig.2: Landscapes in the Darling Downs, Queensland, Australia selected for field work.

Fields

In each of the 3 landscapes, 8 irrigated Bt cotton fields were selected, resulting in altogether 24 fields (Table 1). Fields were selected based on the surrounding crops (or the growers' plans at the time of selection). We selected fields that were neighboured by several other cotton fields, fields that were surrounded by other crops and no other cotton fields, and fields in between to obtain a gradient.

Data on field characteristics were obtained from the growers with questionnaires, including field layout (solid rows: every row planted or double skip rows: two rows planted, one row blank), variety name, seed density per row metre, planting date, defoliation date, date of irrigation events, and product and date of insecticide spray events. In addition, we collected data on geographical coordinates (measured at Northern edge of each field using handheld GPS devices). The final crop height was estimated on the last field trip by measuring the height of the main crop canopy once at each field side and calculating the mean value.

In addition to the cotton fields, 3 pigeon pea refuges next to 3 selected cotton fields were chosen in each landscape, resulting in 9 refuges in total.

Weather

Daily weather data (minimum and maximum temperature, rainfall, maximum wind speed and directionat 9.00 am) were obtained from stations maintained by the Darling Downs Cotton Growers Association and Total Ag Services (Dalby, QLD, Australia). Weather station Nandi was located near field N8, station Horaine in the Cecil Plains landscape near field C1, and station Millmeran in the landscape Pampas near field P7. Due to a malfunction of the Millmeran weather station between 13 Dec and 24 Jan, no data are available for this period. Rainfall data for the whole field season for the Pampas landscape were taken from the weather station Tosari (operated by the Australian Bureau of Meteorology), which is in close proximity to the Millmeran station (near field P7). This station, however, did not record temperature and wind data. In addition, no wind data are available from 1 Dec to 13 Jan from the Cecil Plains weather station.

Helicoverpa spp. egg collection

Helicoverpa spp. eggs were collected in the selected cotton fields on 9-10 Dec, 2009, 6-8 Jan, 19-22 Jan, 9-11 Feb, 23-26 Feb, 2010, and 16-18 Mar, 2010. Whenever possible, collections were done at all 4 sides of each field, which were named according to the most appropriate direction (North, East, South, West). From each field side, a transect of 4 points was sampled at 30m, 60m, 90m, and 120m towards the field centre (Fig. 3). The transect was generally positioned in the middle of the field side, but if this was logistically not feasible, a distance of at least 200m from the adjacent field sides was ensured. In total, *Helicoverpa* spp. eggs were collected at 16 points per field. At each sampling point, *Helicoverpa* eggs were sampled for 5 minutes by visual searching of the plants (Fig. 4). Until the early flowering stage (9-10 Dec and 6-8 Jan), whole plants were searched thoroughly for *Helicoverpa* spp. eggs. Later in the season, examinations focused on the top third of main stems and vegetative side stems of the plants because the vast majority of *Helicoverpa* spp. eggs were shown to be oviposited there (Dillon & Fitt, 1995). Eggs were transferred with a moist fine paint brush to gelatine capsules, size 00 (Paddymelon Gourmet Foods, East Brunswick, VIC, Australia), and the colour of each egg was recorded. At each sampling point, the number of searched plants was recorded for egg density calculation. When egg numbers exceeded 4 eggs per point in several fields, only 2-4 eggs were collected per point and the surplus number of eggs was noted for density calculation. After collection, capsules were stored in a cooled box and upon return to the field station, in an air conditioned room at 25°C. Principle growth stages of cotton were determined for each field according to the BBCH scale (Munger et al., 1998). Stages of neighbouring crops were also recorded as either vegetative, flowering, ripening, or completely dry or harvested.

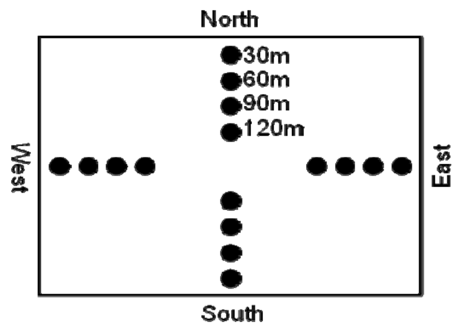

Fig.3: Sampling was done at 16 points within each field, at 30m, 60m, 90m, and 120m from each field edge.

A preliminary egg collection was conducted on 25-27 Nov, 2009 in 6 fields per landscape. Of those fields, 2-3 fields per landscape were included in the final sampling design, while 3-4 fields per landscape were not sampled later on.

In addition to cotton fields, sampling was carried out in pigeon pea refuges next to cotton on 24-27 Nov, 2009, 6-8 Jan, 8-11 Feb, and 16-18 Mar, 2010. The same sampling procedure was followed except that only one field edge was sampled. To get an idea of pest pressure in pigeon pea, the damage level (none, rare feeding traces, frequent feeding traces, 5% of leaf area or of pods damaged, 30% or more of leaf area or pods damaged) and the number of small (<2cm) and large (>2cm) lepidopteran larvae on the searched plants was recorded

Fig.4: *Helicoverpa* spp. egg on cotton square.

After each field trip, hatched larvae were transferred individually with a moist paint brush from the gelatine capsules to plastic trays with circular wells (23mm in diameter, 18mm in height; Tacca Plastics Australia Pty Ltd., Moorebank, NSW, Australia) filled with artificial diet according to Teakle & Jensen (1985) except proprionic acid was substituted for formalin (0.08%). Rearing trays were covered and heat-sealed by a perforated mylar lid (Unipac Solutions, Port Kembla, NSW, Australia). Once all larvae have hatched, the trays were sent via overnight courier to CSIRO Myall Vale (Narrabri, NSW, Australia) for species identification. Ten or more days after each field trip, remaining egg capsules were checked for emerged parasitoids, which were sorted into the groups *Telenomus* spp. (Hymenoptera: Scelionidae) or Trichogrammatidae (Hymenoptera, incl. *Trichogrammatoidea* spp. and *Trichogramma* spp.) (DPI 2003).

Helicoverpa armigera egg cards

In addition to the collection of *in situ Helicoverpa* spp.egg, we exposed *Helicoverpa* spp. egg cards early in the season (7-10 Dec, 2009), mid-season (19-22 Dec, 2010) and late-season (23-26 Feb, 2010) to measure predation and parasitization rates.

For each of the 3 egg card batches, between 600 and 1200 *Helicoverpa armigera* pupae were shipped from CSIRO Myall Vale and placed in groups of 60 in white 5l plastic buckets (People in Plastic, Rocklea, QLD, Australia) with the ground covered with vermiculite. The buckets were closed with a polypropylene hair net (RCR International Pty Ltd, Moorabbin, VIC, Australia). Room temperature was set to 25°C and adjusted to either 22°C or 28°C when necessary to slow down or speed up moth development and egg production, respectively. Once the first moths emerged, they were provided with a solution of 20g honey, 20g sugar, and 2g ascorbic acid per litre water offered in a 50 ml plastic tube. A cotton wick (10cm long, 1cm diameter, Richmond Dental, Charlotte, NC, USA) was pushed through a hole in the lid of the tubes to make the food available for the moths. The honey solution was replaced every 1-2 days when the wick was covered with scales. Moths emerging too early were placed in 10°C to arrest development for up to 5 days. When the first eggs were observed, a fitting green cardbord tube was placed in each bucket and a cardbord disk with several holes for ventilation and light was secured on top. From the evening before the start of the field trip, cardbords were changed twice a day to ensure that eggs were not older than 12h (except for the trip 7-10 Dec, 2009, when cardboards were changed only once a day). Egg cards with 7 to 20 eggs were cut from the cardboards (except for the trip 7-10 Dec, 2009, when some egg cards contained up to 150 eggs), numbered with a pencil, and photographed.

In the field, egg cards were stapled to the underside of a cotton leaf to avoid direct sunlight (Fig. 5). One egg card was used for each of the 4 points at each field side similar to the egg collection procedure described previously (Fig. 3). To avoid predation by ants and other ground dwelling predators, the stem of the cotton plant below the egg card was painted with tangle trap (Australian Entomologica Supplies, Bangalow, NSW, Australia). Leaves and branches around the tangle trap were cleared using secretors. The location of the egg cards was marked with a piece of flagging tape stapled or bound on a plant near the egg card.

Fig.5: *Helicoverpa armigera* egg card stapled on the underside of a cotton leaf after 2 days of exposure to parasitoids and predators.

To be able to assess the importance of ground dwelling predators, a subset of 4 plants per field was fitted with additional egg cards that were applied in the same way except that no tangle trap was used. This was done in 11 fields (7-10 Dec, 2009), 10 fields (19-22 Jan, 2010) and 23 fields (23-26 Feb, 2010).

Furthermore, 24 (7-10 Dec, 2009), 64 (19-22 Dec, 2010), and 91 (23-26 Feb, 2010) egg cards were fixed into 5cm Petri dishes and placed in 10×7 cm organza bags (Ribtex International, Noble Park, Australia) as a control for complete predator and parasitoid exclusion. This treatment was distributed over all fields and allowed to assess if *H. armigera* larvae hatched in the field.

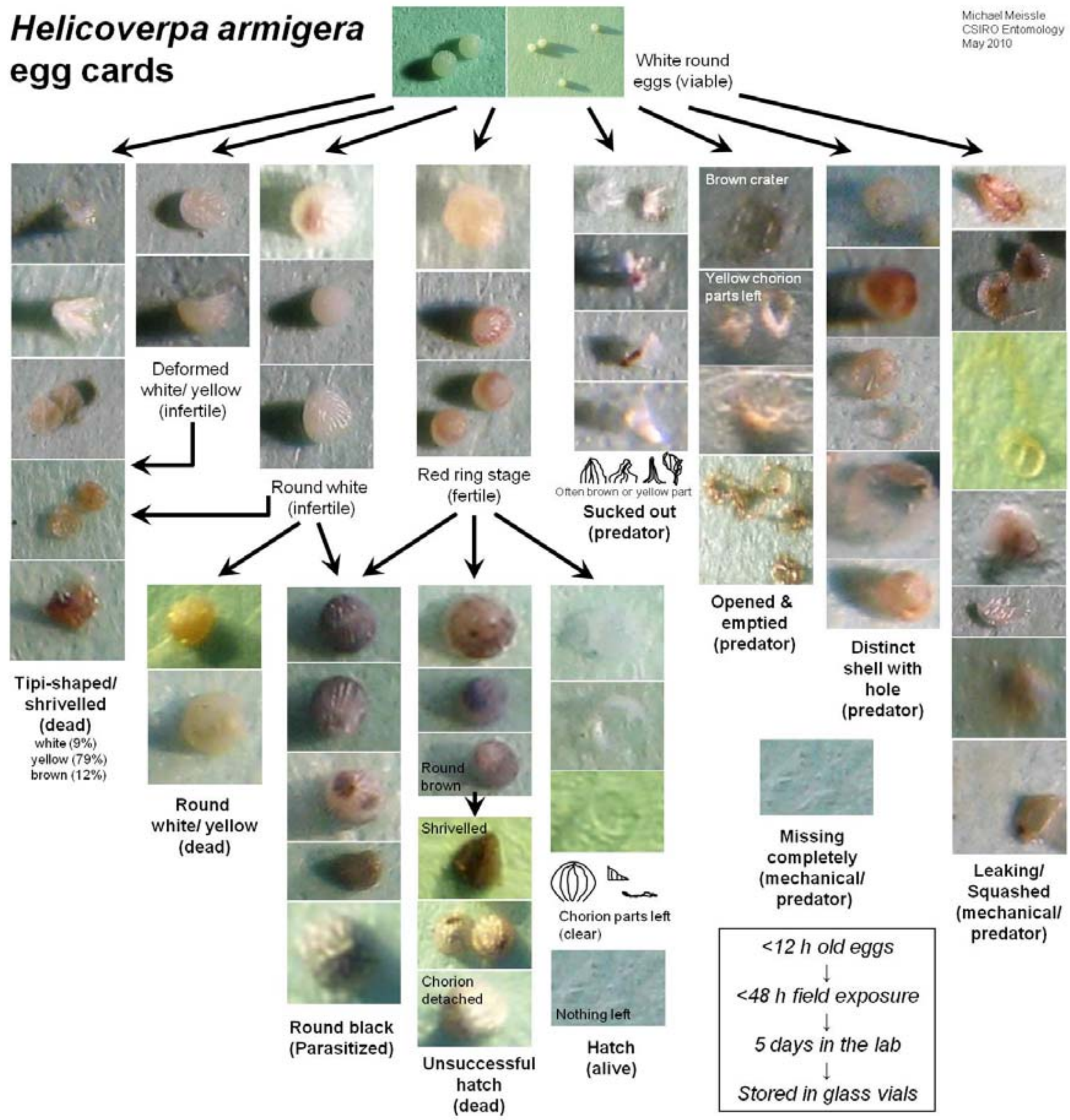

Fig.6: Classification of *Helicoverpa armigera* eggs after 2 days of exposure to parasitoids and predators.

After 39 to48 hours of exposure in the field, egg cards were collected and stored in cooled bags. Back at the field station or laboratory, each egg card was photographed again. Thereafter, egg cards were placed upside down over a water-filled tray so that hatching larvae would silk into the water. After 5 days, all larvae have hatched and a third photograph was taken, before egg cards were stored in glass vials (27 mm diameter, 57mm high) closed with a ventilated lid. After 14 or more days, egg cards on each card were classified according to Fig. 6 using the 3 photographs of each egg card and the microscope. Parasitoids that emerged in the glass vials were counted and transferred to glass specimen tubes (12.8mm diameter, 50mm high) filled with 70% ethanol.

To verify the result of predation, we exposed 1 egg card to an adult green mirid (Heteroptera: Miridae), 2 egg cards to a ladybird larva (Coleoptera: Coccinellidae), and 4 egg cards to an adult *Hippodamia variegata* (Coleoptera: Coccinellidae) for several hours or overnight. The eggs on those cards were classified according to Fig. 6. This small study allowed us to associate eggs classified as ‘sucked out’ and ‘opened & emptied’ with predation.

Mapping

Landscapes were mapped in a circle with 2.5km radius around the centre of each sampled cotton field. Visual mapping was done on 26 Feb, 17 Mar, and 18 Mar, 2010 for the Pampas, Cecil Plains, and Nandi landscapes, respectively. The crops present during the field season were *Bt* cotton, conventional refuge cotton, beans (different types), maize, sorghum, sunflower, and pigeon pea. Crops were recorded with information on the growth stage similar to the neighbouring crops of the sampled cotton fields (vegetative, flowering, ripening, dry/harvested). The growth stages of the plants on each field in the landscape at the other sampling occasions were estimated based on the collected data for neighbouring crops of the sampled cotton fields, on earlier mapping before the fields were selected, and on information obtained from the growers. Recorded landscape features other than crops were leucaena fields (leguminous forage tree), fallow fields, pasture, native vegetation, dams, and buildings (Annex 1).

Maps were created in ArcGIS 9.31 (ESRI, Redlands, CA, USA) for each landscape for each sampling occasion (Annex 1). Aerial images of each landscape (Google Earth Professional 2009, Google Inc., Mountain View, CA, USA) were rectified for the projected special referencing system GDA_1994_MGA_Zone_56. Landscape features were then added as polygons with a corresponding value (Annex 1) for each sampling occasion.

Results

Landscape characteristics

For the presentation of landscape characteristics, the landscape features within a circle of 2.5km radius around each field were summarized into Bt cotton, crops other than Bt cotton, fallow, and other features. Mean values for the 8 fields per landscape were calculated for each sampling occasion. In the Pampas landscape, most fields (>70%) remained fallow until late January 2010 (Fig. 7A, Annex 1). The main crop was Bt cotton with ca. 10%. Other crops (maize, sorghum, and pigeon pea) were present within 2.5km around the Bt cotton fields on less than 5% of the area. Late January, the area of crops other than cotton increased to almost 40%, after many fields were planted to sorghum, maize, and beans. Landscape features other than crops or fallow (pastures, native vegetation, dams, and buildings) made up little more than 10% of the landscape.

In the Cecil Plains area, the majority of fields around the cotton fields remained fallow throughout the season (ca. 65%, Fig. 7B, Annex 1). Bt cotton made almost 15% of the landscape features and crops other than Bt cotton (i.e. maize, sorghum, sunflower, conventional cotton, and pigeon pea) ca. 10% from December 2009 until end of March 2010. Similar to Pampas, landscape features other than crops and fallow land represented little more than 10%.

The percentage of fallow fields was lower in the Nandi landscape (<40%) at the first sampling early December 2009, when mainly young sorghum was present (Fig. 7C, Annex 1). Altogether, the area planted to crops other than Bt cotton was ca. 25% and increased to 30% late January. Bt cotton represented more than 15% of the area. Large areas of pasture, native vegetation, and leucaena plantations (perennial forage legume) resulted in more than 20% features other than crops or fallow.

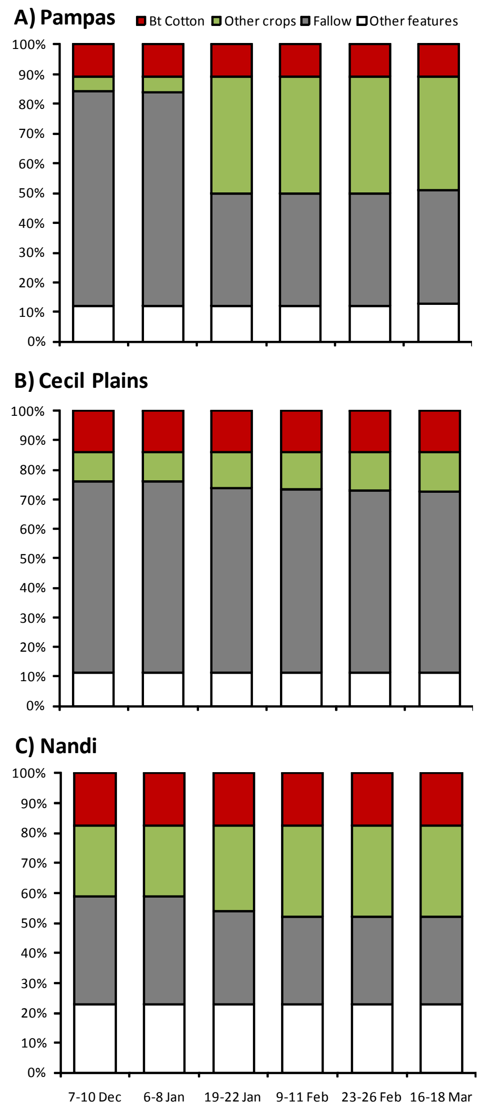

Fig.7: Composition of the A) Pampas, B) Cecil Plains, and C) Nandi landscapes during the sampling season 2009/2010. Values are mean values of the 2.5 km radius circles around each of 8 fields per landscape.

Field characteristics

Cotton fields were either planted solid (every row planted) or laid out in double skip rows (two rows planted, one row empty (Table 1). Solid rows dominated in Pampas and Nandi. The fields in Cecil Plains were half solid, half skip. The seed density ranged between 9 and 17 seeds per row metre.

The dominating variety was Sicot 71 BRF (Bollgard II®, Roundup Ready Flex®, see CSD 2009, Table 1). Growers had chosen also Sicot 70 BRF in Cecil Plains and Sicot 70 BL

(Bollgard II®, Liberty Link®) in Nandi. One field in Nandi and one side of a field in each Pampas and Cecil Plains was planted to the okra leaf variety Siokra V18 BRF. Bollgard II® indicates Bt cotton expressing Cry1Ac and Cry2Ab (Monsanto, St. Louis, USA). Roundup Ready Flex® and Liberty Link® stand for herbicide tolerance traits.

Cotton was planted in the whole month of October 2009 (Table 1). Defoliated occurred between end of February and end of April 2010. At the end of the growing season, the crop canopy was between 75 and 105cm high.

Table 1: Field characteristics of the 24 sampled Bt cotton fields in the Pampas, Cecil Plains, and Nandi landscapes. Geographical coordinates were taken at the Northern edge of each field. Row layout solid means every row planted, skip means 2 rows planted, one row empty. Final crop height in cm at the end of the season (last field trip) estimated based on the mean of one measurement at each field edge.

Field	Latitude	Longitude	Row layout	Seeds/ m row	Variety name	Planting date 2009	Defoliation date 2010	Final crop height [cm]
Pampas								
P1	27.8297S	151.4618E	solid	12	71BRF	29.10	01.04	98
P2	27.8255S	151.4393E	solid	12	71BRF	20.10	01.04	88
P3	27.8170S	151.4316E	skip	10	71BRF	01.10	Early 04	105
P4	27.8204S	151.4002E	solid	16	71BRF	19.10	04*	100
P5	27.8045S	151.3852E	solid	14	71BRF	12.10	01.04	87
P6	27.7991S	151.4032E	solid	16	71BRF*	21.10	03.04	87
P7	27.8526S	151.4428E	solid	12	71BRF	16.10	23.03	98
P8	27.8820S	151.4123E	solid	12	71BRF	22.10	10.04	94
Cecil Plains								
C1	27.5425S	151.2313E	skip	10	71BRF	12.10	End 03	85
C2	27.5539S	151.2124E	skip	10	71BRF	12.10	End 03	87
C3	27.5758S	151.2195E	skip	9	71BRF	03.10	25.02	75
C4	27.5493S	151.2531E	skip	10	71BRF	04.10	12/23.03	79
C5	27.5870S	151.2858E	solid	13	71BRF	01.10	19.03	76
C6	27.5974S	151.2633E	solid	12	71BRF#	29.10	30.03	93
C7	27.5791S	151.2603E	solid	10	70BRF	17.10	28.03	91
C8	27.6203S	151.2442E	solid	14	70BRF	30.09	23.03.	86
Nandi								
N1	27.3344S	151.1875E	solid	14	71BRF	25.10.	29.03.	78
N2	27.3300S	151.1632E	solid	14	70BL	25.10.	07.04.	96
N3	27.3306S	151.1543E	solid	14	70BL	27.10.	07.04.	93
N4	27.3179S	151.1640E	skip	13	V18BRF	25.10.	01.04.	97
N5	27.2970S	151.1782E	solid	13	71BRF	22.10.	26.03.	78
N6	27.2940S	151.1930E	solid	14	71BRF	24.10.	20.03.	93
N7	27.2982S	151.1999E	solid	14	71BRF	24.10.	20.03.	69
N8	27.2647S	151.1579E	solid	17	71BRF	16.10.	29.04.	94

* Northern 115 rows planted to V18BRF
# Western 110 rows planted to SV18BRF

Most fields needed to be irrigated at planting time because no suitable planting rain occurred. After that, growers irrigated their cotton fields 2-3 times, depending on water availability (Table 2). One field in Nandi was irrigated only half once and one field in Cecil Plains did not receive postplanting irrigation at all due to lack of water. After a good rain in the South of the Nandi landscape, 2 fields received a fourth irrigation.

Broad spectrum insecticides were applied to the Bt cotton crops mainly to control sucking pests, such as mirids and aphids. The active ingredients used were fipronil (phenylpyrazole), dimethoate (organophosphate), and clothianidin (neonicotinoid, Table 2). While no spraying was necessary in 2 fields in Pampas, farmers applied insecticides 1-4 times in all other fields.

Table 2: Post planting irrigation events and insecticide applications on the the 24 sampled Bt cotton fields in the Pampas, Cecil Plains, and Nandi landscapes in the season 2009/2010. Applied insecticides were fipronil (Fip, Regent® 200SC, phenylpyrazole), dimethoate (Dim, Rogor®, organophosphate), and clothianidin (Clo, Shield®, neonicotinoid).

Field	Irrigation events [Dates]	Insecticide Application [Date (active ingredient)]
Pampas		
P1	12.12., 25.01., 22.02.	13.01., 05.02. (Fip)
P2	15-17.01., 08.02.	24.12., 05.02. (Fip)
P3	14-22.12., 19-22.01.	10.11., 06.01. (Dim), 03.12., 26.12. (Fip)
P4	29.12., 21.01., 08.02.	No spray
P5	22.12., 25.01.	22.01. (Dim)
P6	14-17.01., 4-6.01./10.02.	No spray
P7	10-15.12., 16-21.01.	09.01. (Fip)
P8	28.12., 21.01., 20.02.	14.12. (Dim)
Cecil Plains		
C1	20.12., 21.01.	12.12., 23.02. (Dim)
C2	20.12., 21.01., 26.02.	10.12., 22.01., 03.02. (Dim)
C3	No irrigation	End 01 (Clo)
C4	10/20.01., 10.02.*	End 01 (Clo)
C5	16-31.12., 14.01.	16.01. (Dim)
C6	20.12., 14.01., 05.02.	23.12. (Fip), 27.02. (Clo)
C7	16.12., 12.01., 01.02.	23.12. (Fip), 27.02. (Clo)
C8	15.12., 07.01., 25.01.	22.01. (Fip)
Nandi		
N1	24.12., 15.01., 30.01., 27.02.	05.12., 14.01. (Fip)
N2	27.12., 18.01., 02.02.	15.12., 12.01. (Fip)
N3	27.12., 18.01., 02.02., 02.03.	15.12., 12.01. (Fip)
N4	24.12., 15.01., 05-07.01.	05.12., 14.01., 13.02. (Fip)
N5	28.12., 20.01.*	11.12. (Dim), 20.01., 22.01. (Fip)
N6	29.12., 30.01.	30.01. (Fip)
N7	10.01.*	30.01. (Dim)
N8	17.12., 17.01., 14.02.	27.01. (Fip)

* only part of field irrigated

According to information from our collaborating growers, conventional cotton needed 4 sprays against *Helicoverpa* spp. over the whole season. Sorghum was sprayed once or twice with a nucleopolyhedrovirus formulation (Gemstar® or Vivus Max®) and/or once or twice with pyrethroid (e.g. Dominex®) against *Helicoverpa* spp. at flowering time. Some sorghum crops, however, did not need any insecticide spraying. Maize was generally not sprayed in the 2009/2010 season. Sunflowers received one pyrethroid spray against *Helicoverpa* spp. before and at flowering. Mungbeans needed several treatments with insecticides, including pyrethroids, dimethoate, indoxacarb (e.g. Steward®).

Weather

A maximim temperature of more than 40°C was reached early December, when the first batch of egg cards were exposed and the first egg collection was done (Fig. 8). The average temperature (maximum temperature + minimum temperature / 2) was also highest during the first sampling at 30°C. The lowest recorded temperature was 10°C mid January. The

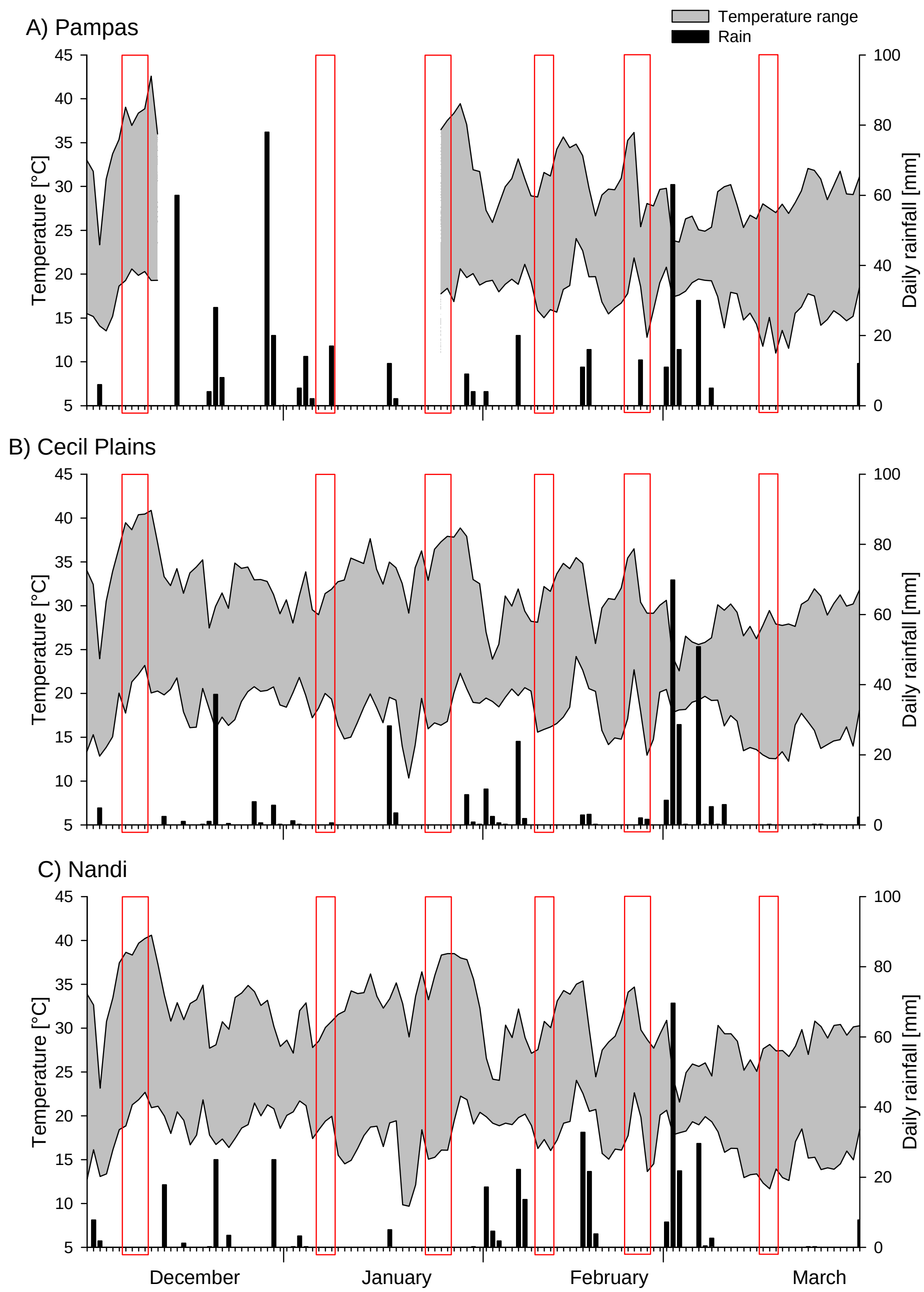

Fig. 8: Daily maximum and minimum temperatures in °C and daily rainfall in mm for the landscapes A) Pampas, B) Cecil Plains, and C) Nandi during the sampling period 2009/2010. Sampling trips are indicated with red boxes. Temperature data between 13 Dec and 24 Jan for Pampas are missing due to malfunction of weather station.

temperature decreased from February onwards. During field trips 2-5, the average temperature was 23 to 25°C. The lowest average temperature was recorded during the last sampling mid March at 20°C.

Rainfall was sporadic and very local (Fig. 8). According to growers´ information, it was common that some fields received considerable rain of more than 60mm, while other fields on the same farm received very little. In Nandi and Cecil Plains, rain events were scarce early in the season and increased in February and early March with a maximum recorded on 2 March with 63-70mm. In Pampas, significant rainfall was also recorded in the second half of December and early January with a maximum of 78mm on 29 Dec.

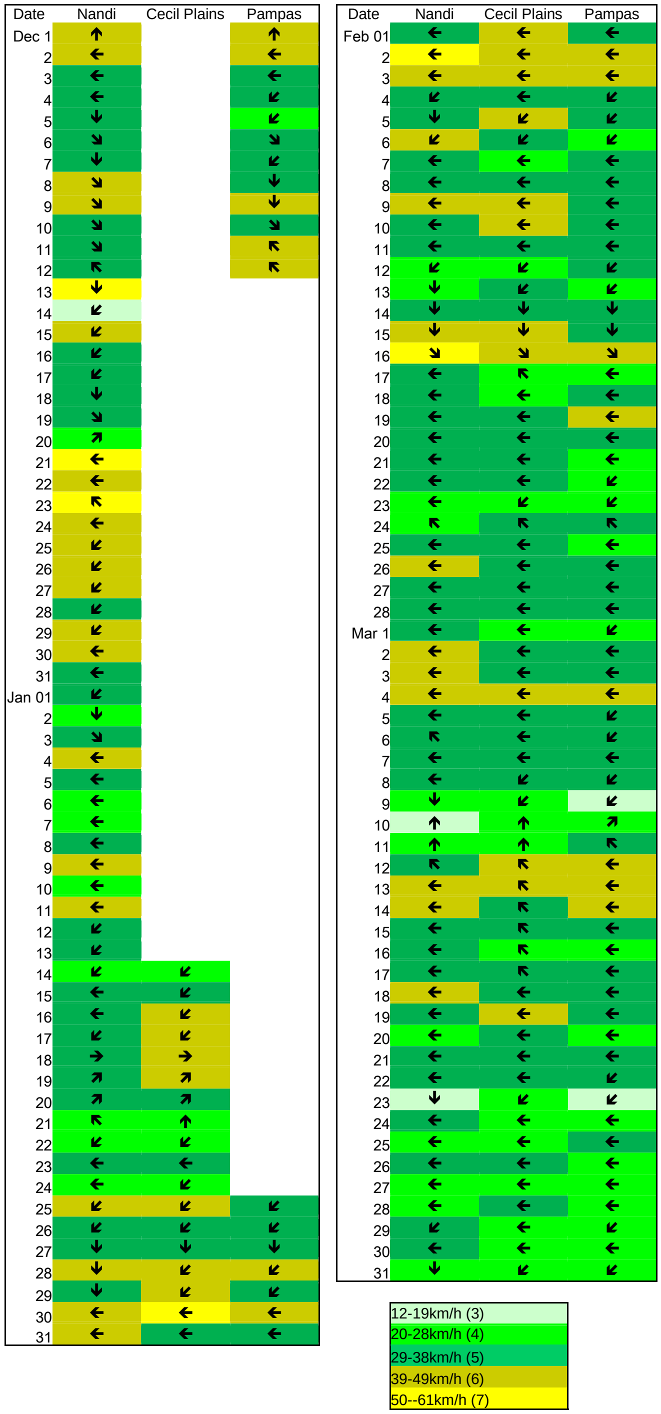

Fig. 9: Daily maximum wind speed and direction for the landscapes Pampas, Cecil Plains, and Nandi during the sampling period 2009/2010. Missing data for Cecil Plains and Pampas due to malfunction of the weather stations. Wind speeds are colour coded and classified according to the Beauford scale (Beauford number in paranthesis in the legend). Wind directions are indicated with arrows (🡫 = wind originating from North)

The predominant wind direction was East, ranging from North to Southeast (Fig. 9). Winds blowing from South or West were observed only occasionally. The highest recorded maximum daily wind speed was 60km/h (Gale force), but usually maximum wind speeds were between 20 and 50 km/h (Breeze).

Helicoverpa spp. egg collection

*Population dynamics of Helicoverpa spp.*and egg parasitoids

All eggs found in one field were divided by the total number of plants sampled in the field to obtain the egg density per field. Mean *Helicoverpa* spp. egg densities per cotton plant at the preliminary field trip 25-27 November were 0.4 ± 0.10, 0.6 ± 0.77, and 0.6 ± 0.50, in the landscapes Pampas, Cecil Plains, and Nandi, respectively, when cotton plants were at an early

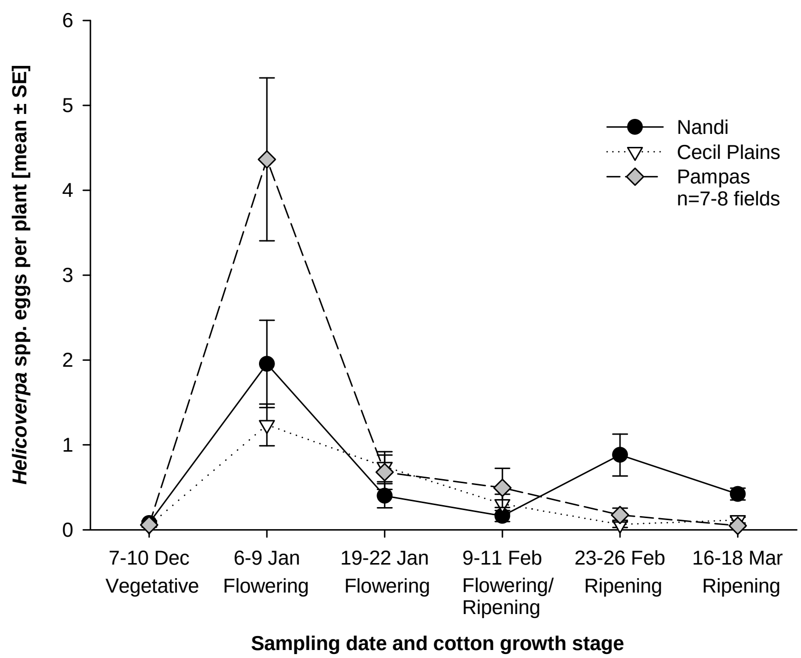

Fig. 10: Mean *Helicoverpa* spp. egg densities per cotton plant during the field season 2009/2010 summarized for the landscapes Nandi, Cecil Plains, and Pampas.

vegetative stage. Egg densities dropped below 0.1 mid December, and increased dramatically early January, when cotton was flowering (Fig. 10). Highest numbers of eggs were found in Pampas and almost 5 times lower numbers in Cecil Plains. Thereafter, densities declined constantly towards the end of the season with the exception of Nandi, where a second peak occurred end of February at the ripening stage (boll development).

This general pattern is also apparent when looking into the data for each individual cotton field (Fig. 11). Variation between fields, however, was high.

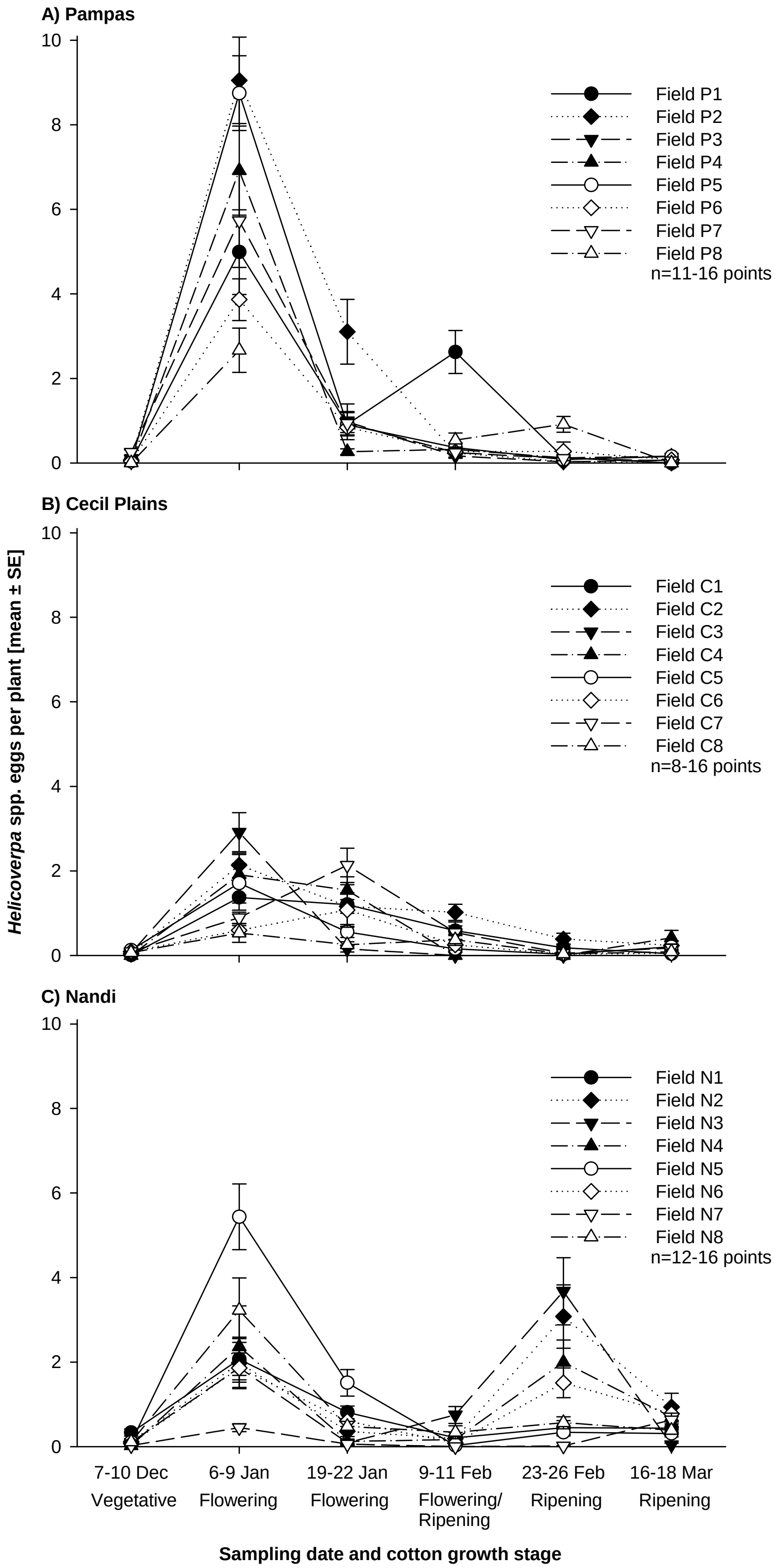

Fig. 11: Mean *Helicoverpa* spp. egg densities per cotton plant during the field season 2009/2010 for each field in the landscapes A) Pampas, B) Cecil Plains, and C) Nandi.

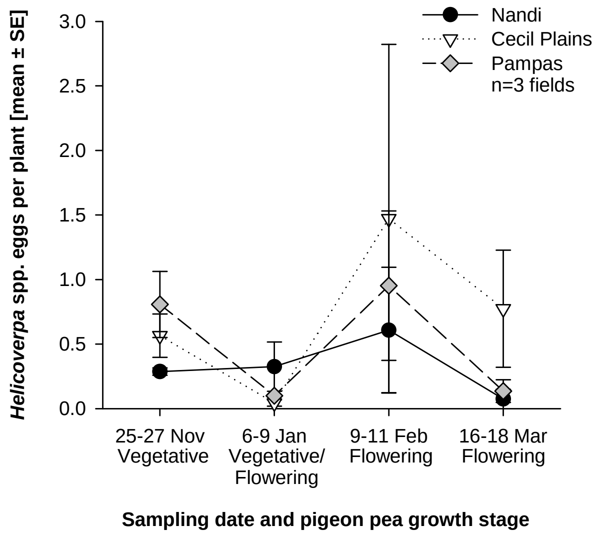

Fig. 12: Mean *Helicoverpa* spp. egg densities per pigeon pea plant during the field season 2009/2010 summarized for the landscapes Nandi, Cecil Plains, and Pampas.

Late November, when the preliminary field trip was carried out, *Helicoverpa* spp. egg densities in the pigeon pea refuges were similar to those in cotton fields with 0.3-0.8 eggs per plant (Fig. 12). In contrast to cotton, however, egg numbers dropped early January to less than 0.5 eggs/plant, when only some pigeon pea fields started flowering. Increased egg densities were observed early February and numbers decreased again mid March.

Parasitoids were not found in any sample taken before January (Fig. 13). Parasitization rates were calculated for field in which more than 5 *Helicoverpa* spp. eggs were collected. From early January until the end of the season parasitization rates in cotton were at a relatively high level of 40-80%. Parasitism reached a peak early February, except for the Nandi landscape, where the rates decreased from late January and reached the highest level mid March.

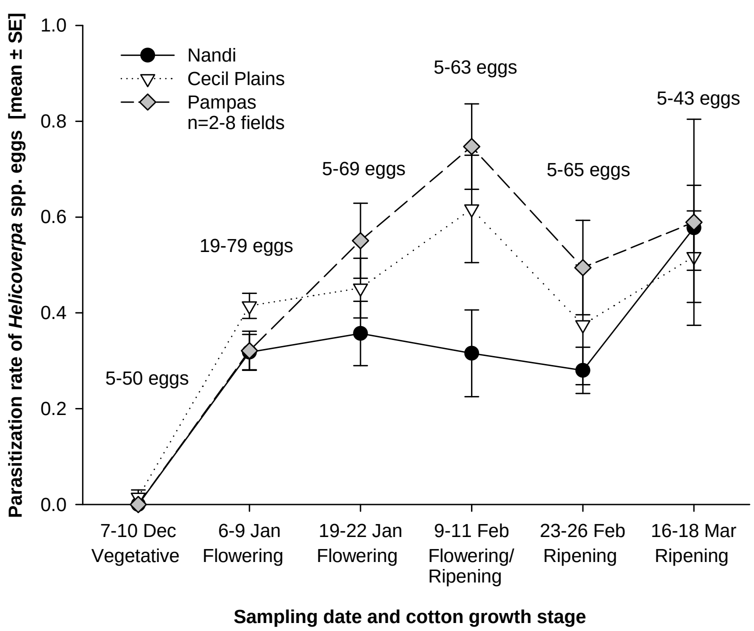

Fig. 13: Mean parasitization rates of *Helicoverpa* spp. eggs collected in cotton during the field season 2009/2010 summarized for the landscapes Nandi, Cecil Plains, and Pampas. Field in which less than 5 eggs were collected per trip were excluded.

Data on parasitization rates of each individual cotton field reveal little information except the fact that variation is very high (Fig. 14). Many datapoints are missing because less than 5 eggs were collected in the corresponding fields.

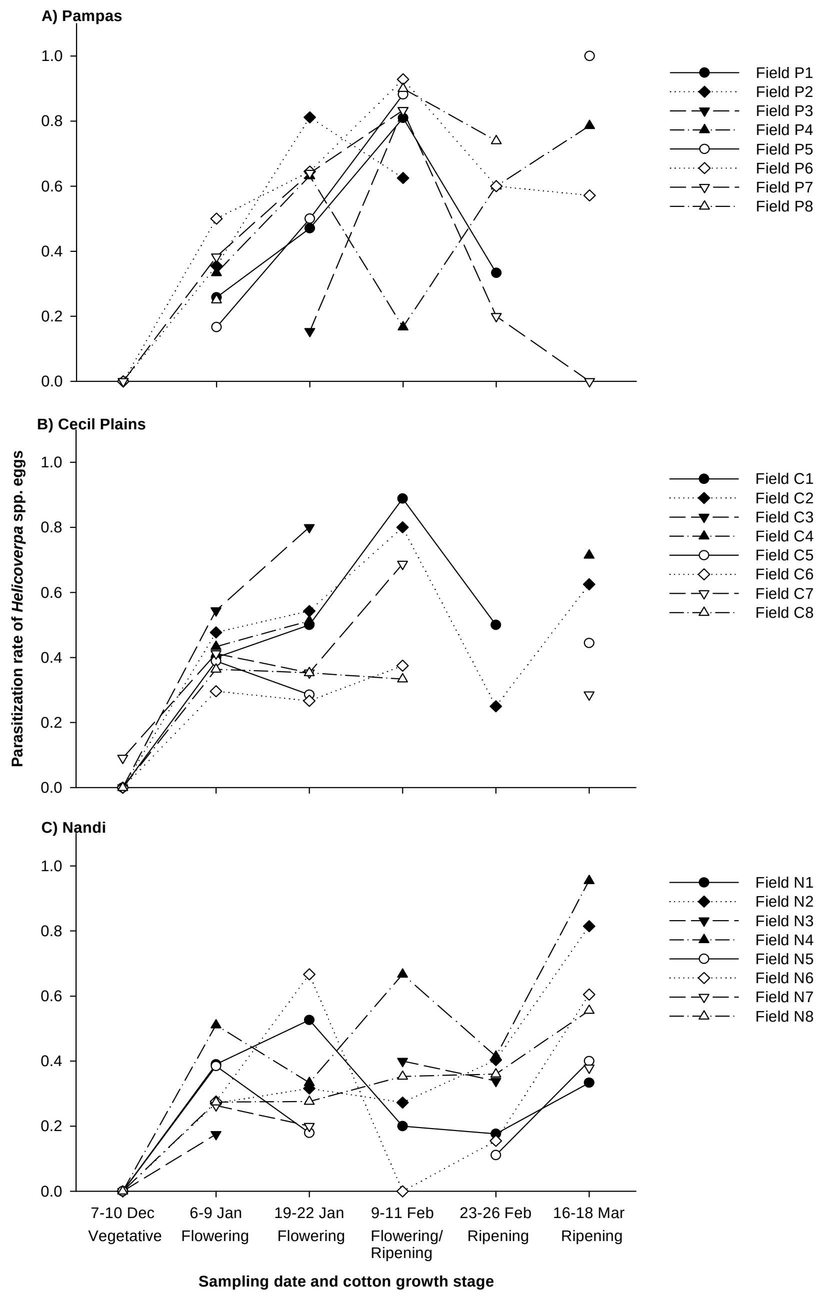

Fig. 14: Mean *Helicoverpa* spp. egg parasitization rates in cotton during the field season 2009/2010 for each field in the landscapes A) Pampas, B) Cecil Plains, and C) Nandi. Data points with less than 5 eggs were excluded.

In pigeon pea, parasitization rates could be calculated for 19 samples with 5 or more eggs collected for the 4 trips altogether. All of those samples were 0 with the following exceptions: Cecil Plains 9-11 Feb (36%) and 16-18 Mar (5%), Nandi 6-9 Jan (14%), and Pampas 9-11 Feb (17%).

Species composition of Helicoverpa spp. and egg parasitoids

During the preliminary field trip 25-27 November, more than 75% of the collected eggs in cotton were *H. punctigera*. Similar values were recorded in the first field trip early December (Fig. 15). In contrast, species composition shifted to more than 80% *H. armigera* early January. Subsequently, *H. armigera* lost importance until *H. punctigera* again dominated the collection at the end of the season.

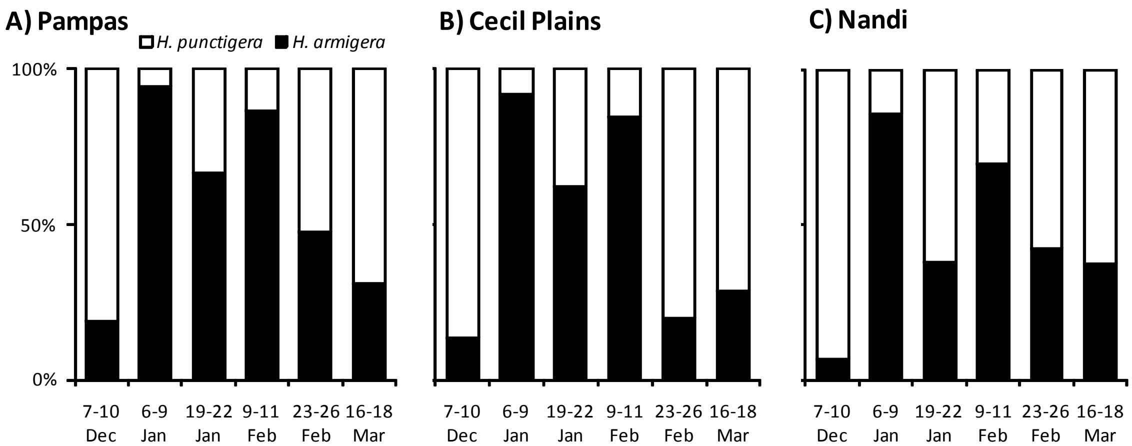

Fig. 15: Species composition of *Helicoverpa* spp. in the landscapes A) Pampas, B) Cecil Plains, and C) Nandi. The percentage was calculated after all determined individuals of each species were summarized per landscape.

In pigeon pea, the species composition of *Helicoverpa* spp. was similar to cotton. In the Pampas landscape, *H. punctigera* dominated in November and March with ca. 60%, while *H. armigera* was the only species determined in February. No hatching eggs were available from Pampas for January. In Cecil Plains, only 11% *H. armigera* were collected in November, but 100% in the subsequent 3 collections. Nandi, however, showed 75-88% *H. armigera* in November, January, and March, but *H. armigera* dominated in February with 66%.

Parasitoids were dominated by Trichogrammatidae (*Trichogramma* spp. and *Trichogrammatoidea* spp.). For cotton, the mean number (all eggs with emerging Trichogrammatidae pooled) of parasitoids emerging from each egg was 2.3 individuals. From most eggs, 2 (56%), 3 (33%), or 1 (10%) parasitoids emerged. In rare cases, even 4 (1.5%) or 5 wasps (0.1%) emerged. Low numbers of *Helicoverpa* spp. eggs parasitized by *Telenomus* spp. were collected in cotton, but only during the two field trips in January. Early January, *Telenomus* spp. were found in 5.4% (4 individuals), 1.0% (2 individuals), and 6.7% (7 individuals) of the parasitized eggs in Pampas, Cecil Plains, and Nandi, respectively. Late January, three individuals were recorded in cotton, one in Pampas (0.8% of parasitized eggs) and 2 in Cecil Plains (2.1%). From eggs parasitized by *Telenomus* spp., no multiple individuals emerged.

From pigeon pea, Trichogrammatidae emerged from altogether 14 eggs, while no *Telenomus* spp. were found. Similar to cotton, a mean number of 2.6 individuals emerged per egg.

Within-field differences in Helicoverpa spp. egg densities and parasitization rates

We compared *Helicoverpa* spp. egg densities and parasitization rates within the fields to check if there is a general influence of cardinal direction and if there are edge effects. Kruskal-Wallis ANOVA indicated that there is no difference between the 4 edges (N, E, S, W) of the fields in any landscape at any sampling occasion ($p>0.05$) for both egg densities

(Annex 2A) and parasitization rates (Annex 2B). Similarly, egg densities (Annex 2C) and parasitization rates (Annex 2D) were not statistically different for samples collected at 30m, 60m, 90m, and 120m, indicating that no apparent edge effect occurred (Kruskal-Wallis ANOVA, $p>0.05$).

Influence of field size on Helicoverpa spp. egg densities and parasitization rates

Samples were collected in cotton fields of different sizes. We analyzed if field size influenced *Helicoverpa* spp. egg densities and parasitization rates using Spearman rank order correlations. Even though field size was correlated significantly with egg density (Annex 3A) or parasitization rates (Annex 3B) in some cases, there was no general relationship evident.

Influence of surrounding cotton on Helicoverpa spp. egg densities and parasitization rates

From the ArcGIS maps, we analysed the percentage of cotton area surrounding each of the sampled fields in circles of 0.5, 1.0, 1.5, 2.0, and 2.5km around the centre of the field. The correlations of the cotton area with the *Helicoverpa* spp. egg densities (Annex 4A-4C) and parasitization rates (Annex 4D-4F) were then calculated and plotted for each landscape, circle, and field trip. Even though some correlations were significant, no general trend was evident.

Influence of surrounding non-cotton crops on Helicoverpa spp. egg densities and parasitization rates

From ArcGIS maps, the area planted to crops other than cotton was summarized. Because all crops grown in the analysed landscapes can potentially host *Helicoverpa* spp., we assumed that all non-Bt cotton crops can act as potential sources for *Helicoverpa* spp. We analysed the non-cotton crop area surrounding each of the sampled fields in circles of 0.5-2.5km around the centre of the field. The correlations of the potential source crop area with *Helicoverpa* spp. egg densities (Annex 5A-5C) and parasitization rates (Annex 5D-5F) were calculated and plotted for each landscape, circle, and field trip. Even though some correlations were significant, no general trend was evident.

Helicoverpa armigera egg cards

The control egg cards (protected from predators and parasitoids) revealed hatching rates between 7% in Nandi and 17% in Cecil Plains for the first trip in December, 2009. In January, 2010, a higher hatching rate of 50-53% was observed for the 3 landscapes. In the last trip in February, 33-44% of the eggs hatched from the control cards. Missing eggs were rare with a maximum of 2% in Nandi in February. For the unprotected egg cards, missing eggs were consequently considered as predated. Squashed (leaking) eggs were below 3% for all trips and landscapes on the protected egg cards and similarly low values were recorded on the egg cards exposed to predators and parasitoids. We concluded that squashed eggs were a result of handling rather than of predation and excluded them from analyses.

On the first egg card trip early December, 2009, most eggs on the unprotected cards with tangle trap either died (47-56% summarized for landscapes) or were predated (32-43%) (Fig. 16). Approximately half of the predated eggs were sucked out and half were completely removed from the egg card. Hatching rates were generally low (8-10%) and only 1 egg card was parasitized. The 3 landscapes showed a similar pattern. Mid January, parasitization was very common with lowest values in Cecil Plains (15%) and highest values in Pampas (43%). Predation, however, decreased to 8-15% with ca.2/3 missing eggs. Dead eggs ranged between 32 and 40% and hatching rates were 14-31%. On the last egg card trip in February, parasitization decreased strongly to 1-5%. In contrast, predation increased to 15-35%. Two thirds of the predated eggs were sucked out and 1/3 missing. The proportions of dead and hatching eggs were similar to the last trip at 38-48% and 15-33%, respectively (Fig. 16).

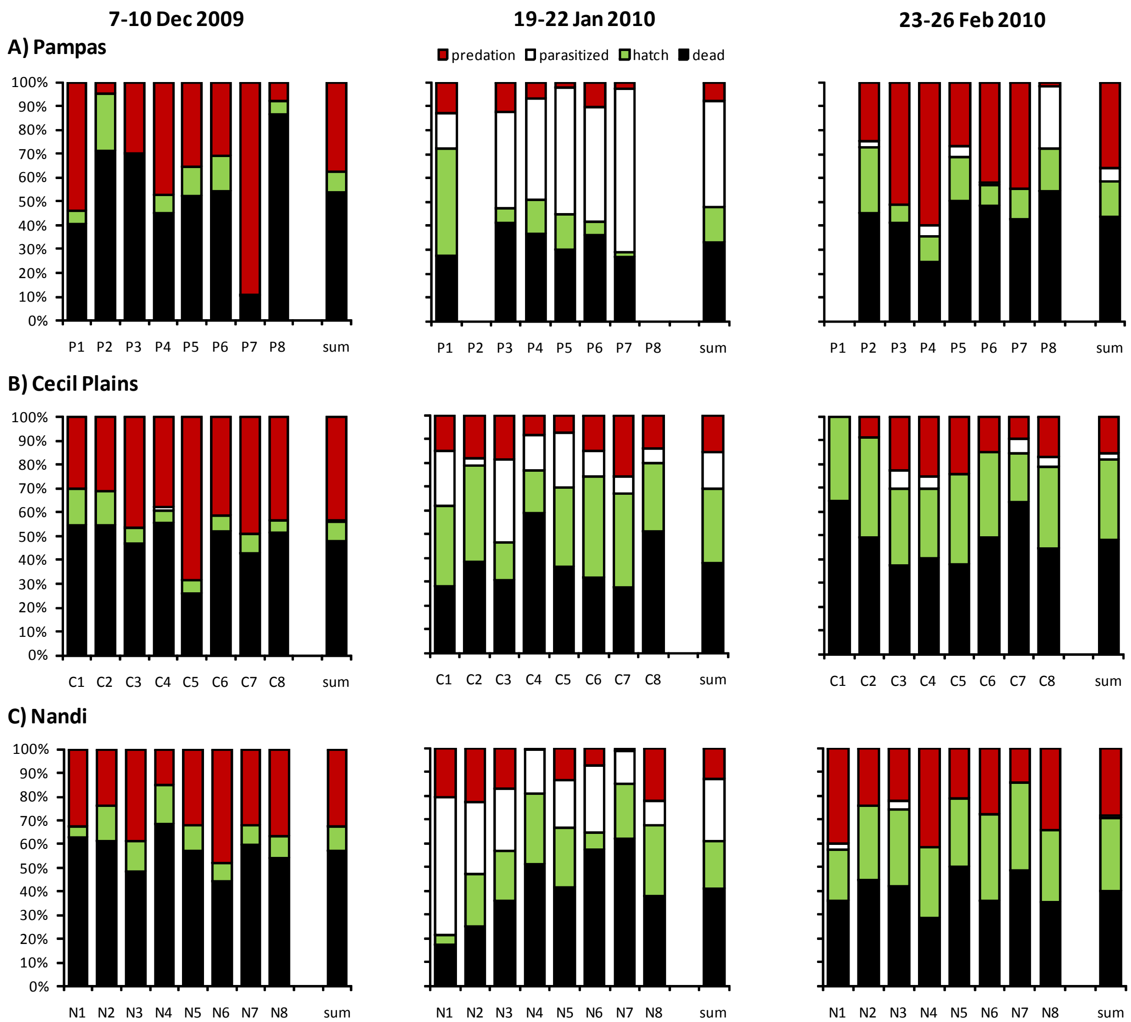

Fig. 16: *Helicoverpa armigera* egg cards exposed to cotton fields for ca. 2 days in December, 2009, January, and February, 2010. Values for each field are means of 16 egg cards. Sum bars indicate means of the 8 fields per landscape.

In addition to the egg cards with tangle trap to exclude ground dwelling, non-flying predators, we exposed a subset of egg cards without tangle trap to allow access for all predators to the egg cards. As expected, more eggs were removed from the egg cards. This resulted in a total predation of 66 and 45% in Cecil Plains and Pampas, respectively, in December, and 32 and 36% in January. No egg cards without tangle trap were exposed in Pampas in December and January. In February, predation was 57%, 38%, and 60% in Pampas, Cecil Plains, and Nandi, respectively. Most of those values are approximately double the predation proportions with tangle trap.

Discussion

Cotton cropping in the season 2009/2010

The composition of crops in the Darling Downs is depending on the two major factors water availability and market price. Water for irrigation is collected from rainfall and stored in dams or pumped from bores. Allowances for bore water are strictly regulated. Consequently, fields that can be irrigated are located around the dams and bores, while the rest of the agricultural fields are dryland that depend on rainfall alone. With the given market price, most growers preferred to grow cotton in their irrigated fields in the 2009/2010 season.

Rainfall dominated the planting date and choice of crops for the dryland fields. In the southern half of the Nandi landscape, good rainfall (ca. 100ml) occurred mid November (according to growers' correspondence).Subsequently, growers in this region planted sorghum on many dryland fields (Annex 1) and also some dryland cotton. In addition, a hailstorm hitting the northern part of the landscape resulted in several lost cotton crops. Growers replaced those crops mainly with irrigated maize. Those events resulted in a relatively high proportion of crops other than cotton compared to the other landscapes early in the season (Fig. 7). In the Pampas landscape, rainfall suitable for dryland farming did not occur until late December and early January (Fig. 8). Then, however, many fields were planted to sorghum and to a lesser extend also maize and beans (Annex 1). In Cecil Plains, no good rainfall happened until the end of the summer season in March (Fig. 8). Consequently, the percentage of crops other than irrigated Bt cotton remained low and most fields remained fallow throughout the season (Fig. 7). The vast majority of growers planted 5% pigeon pea as refuge crops, but individual cases of unsprayed sorghum, maize, or cotton, as well as 100% sprayed cotton were observed.

Water availability also dominated the field layout, defoliation date, and final crop height. Less water is necessary with a skip row layout (2 rows planted, 1 row blank) compared to a solid field. When not enough water was available even though irrigation was warranted, plants remained small with less bolls, which opened earlier in the season (see field C3 and N7, Tables 1,2). Most growers, however, were able to irrigate their fields 2-3 times during the season.

Helicoverpa spp. population dynamics on landscape scale

Helicoverpa egg densities were relatively high both in small vegetative cotton and pigeon pea plants when we carried out the preliminary field trip late November. Those eggs might have been laid by the first generation of moths, which either migrated from inland areas (mainly *H. punctigera*) or emerged from diapauses (mainly *H. armigera*). We confirmed that *H. punctigera* is dominating early season *Helicoverpa* populations in eastern Australia (Fig.15; Zalucki et al. 1986, Baker et al. 2008). After very few *Helicoverpa* spp. eggs were found in vegetative cotton early December, numbers were high early January (Fig. 10). Two factors might have contributed to this: a) moths of the second generation emerged from non-cotton crops and searched for host plants suitable for oviposition, and b) flowering cotton is particularly attractive for *Helicoverpa* spp. for oviposition (Firempong & Zalucki 1990). *Helicoverpa armigera* was the dominating species early January. Later in the season, populations decreased continuously with the exception of a peak in Nandi at the end of February (Fig. 10). Assuming that recruitment from Bt cotton fields is nearly 0, eggs found later in the season need to come from *Helicoverpa* spp. moths emerging from crops other than Bt cotton. Most crops can be potential source crops, even though recruitment differs from crop to crop. Unsprayed pigeon pea, sorghum and conventional cotton yielded highest numbers of living pupae in a survey of several cotton growing regions between 1996-2003, while unsprayed maize and sprayed conventional cotton showed lower numbers (Baker et al. 2008). When crops are sprayed with insecticides (which was the case for some sorghum, beans, and sunflower crops in our study), recruitment is likely to be lower. In Cecil Plains, the lack of crops other than Bt cotton throughout the season might have lead to the decreasing *Helicoverpa* spp. populations observed. Similarly, egg densities decreased in Pampas, probably because alternative hosts have been planted relatively late (late December/ early January). Sorghum, maize, and beans are most attractive for egg laying at the flowering stage, which did not happen in Pampas until early February. Consequently, recruitment of the next generation from those crops occurred when cotton plants started to dry after our last sample. In the Nandi landscape, however, many sorghum crops were planted in November and flowering early January. The peak observed mid February (Fig. 10) in this landscape is thus likely to be a result of third generation moths recruited from those crops. Interestingly, *Helicoverpa punctigera* showed increased proportions in the samples again towards the end of the season. Most authors reported that *H. punctigera* populations remained low until the end of the season (Maelzer & Zalucki, 1999; Zalucki et al., 1986).

Parasitization rates and predation on landscape scale

Even though *Helicoverpa* spp. eggs were abundant early in the season, egg parasitoids were not present until early January. For the prevention of pest outbreaks, however, early presence of natural enemies is crucial. Probably the late population build-up of parasitoids is responsible for a low efficacy to control *Helicoverpa* spp. in the Darling Downs. Relatively high parasitization levels between 30 and 80% until the end of the season, however, may have contributed to the decreasing populations of *Helicoverpa* spp.

The *Helicoverpa armigera* egg cards revealed highest parasitization rates late January. Similar to the field collected eggs, Pampas showed the highest rates (Fig. 13, 16) with 43%. In February, however, parasitization rates for the egg cards were rather low at 1-5%. This is in contrast to the field collected eggs, where rates remained high until the end of the season.

Only very few parasitized eggs were found in pigeon pea, indicating that this crop is a poor source of parasitoids. This has been suggested previously (DPI 2003).

Trichogrammatidae clearly dominated the guild of egg parasitoids, while *Telenomus* spp. were rare and only occurring in January. The biological control potential of Trichogrammatidae is thus higher than for *Telenomus* spp.

An estimate of predation was obtained by the egg cards. Predation was important early in the season and late in the season, when 32-43% and 15-32% of the eggs, respectively, were taken away, sucked out, or opened and emptied (Fig. 16). Interestingly, in January, when parasitoids were highly abundant, predation was rather low. Exclusion of ground dwelling predators resulted in approximately 50% lower predation rates compared to plants without tangle trap. Ground dwelling predators of relevance are mainly ants. Other studies in dryland crops experienced that predation by ants was huge and most egg cards were emptied within the time of exposure if ants were not excluded (B. Walters & F.J.J.A. Bianchi, personal communication). Thus predation by ants can be considered as moderate in our irrigated cotton fields.

Helicoverpa spp. populations and parasitization within landscapes

First, we analysed if there were systematic differences in Helicoverpa spp. egg densities and parasitization between the different sampling point within the fields. Effects of cardinal direction could be present because *Helicoverpa* spp. adults and parasitoids might use wind systems to move between fields. Wind was mostly blowing from the East during the sampling period. Therefore, if movement with the wind was true for short distance movements, moths and parasitoids might have enter the sampled cotton fields and oviposited mainly from the eastern edge. This, however, was not observed (Annex 2A,B). In addition, edge effects might be possible, for example if moths or parasitoids oviposit shortly after entering the field rather than after dispersing throughout the field. This, however, was also not observed (Annex 2C,D). We further looked into the correlation of field size with egg densities and parasitization rates, because a) samples in smaller fields were in closer proximity than in bigger fields, which could result in differences in variation, and b) field size might influence moths or parasitoids. However, field size did neither correlate with egg densities and parasitization rates nor influence the variation in the data (Annex 3).

In a next step, we looked into the crops surrounding our focus fields by mapping the landscape in circles around the centre of the fields. The smallest circle was 0.5km, which included little more than the cotton field itself. The largest circle was 2.5km. When selecting the cotton fields, we aimed at finding fields surrounded by many other cotton fields and little other crops and those surrounded by many other crops and little other cotton. Annex 4 and 5 show that the gradient between fields gets smaller when circles get bigger. Especially in the Cecil Plains landscape, Bt cotton fields were almost equally distributed and very few non-cotton crops were present. Therefore, there is almost no gradient, which makes the correlations meaningless. The situation is better in Pampas and Nandi, even though the gradients are not big. There is no general pattern evident for the performed correlations on egg density and parasitization rates with cotton and potential source crop area. This indicates that either a) the area of cotton (sink) and potential source crop in an area of up to 2.5km around a field does not influence egg densities and parasitization rates, because population dynamics are influenced by factors on a larger scale, or b) the gradient obtained in the present study was not enough to see effects because the areas surrounding the cotton fields were too similar to each other.

Conclusions

Our first research hypothesis is that Bt cotton functions as a sink for *Helicoverpa* spp. and other crops as potential sources. We expected that recruitment is low in areas with a high percentage of Bt cotton and a low percentage of potential source crops, which leads to reduced *Helicoveropa* spp. populations later in the season. Our data support this hypothesis, because *Helicoverpa* spp. populations decreased in the second half of the season in Cecil Plains and Pampas, where few potential source crops were present and the percentage of Bt cotton was high. In contrast, populations increased in Nandi, where potential source crops were present early in the season. Effects of Bt cotton and potential source crop area within a landscape were not evident, either because population dynamics and moth movement occurred on a scale larger than 2.5km, or because the gradient between fields was too small.

Our second hypothesis is that natural enemies of *Helicoverpa* spp. are unaffected by Bt cotton. In this project, we showed that parasitization rates of field collected *Helicoverpa* spp. eggs remained high once parasitoid populations were established early January, which supports the hypothesis. Parasitization rates of sentinel eggs exposed to the fields for 2 days were high in January, but low in February in all landscapes. Predators, however, showed a contrasting pattern, with high predation rates in December and February, and low predator activity in January. Similar to *Helicoverpa* spp. egg densities, effects of Bt cotton and potential source crop area within a landscape were not evident.

In conclusion, our data indicate that Helicoverpa spp. populations are affected by surrounding crops on landscape scale, but within landscape effects were not evident. Natural enemy populations could not be linked to between or within landscape differences.

Outlook

The presented data derived from the field season 2009/2010 only. Field data are known to be highly variable. To be able to see general trends, data from several field seasons are necessary. We were able to obtain a second year of funding from Cotton, Catchments, Communities CRC, and the Grains Research and Development Corporation (GRDC). The former organisation is a multidisciplinary research program around Australian cotton research, and the latter is the research organization financed by the Australian grain growers. We were able to recruit Dr. Cate Paull as a postdoc with experience in landscape-level experiments on pests and beneficial. Fields will be selected in November 2010 in the same landscapes and data will be collected in a similar way to allow direct comparison with the 2009/2010 season. Once more, both egg collections and egg cards will be used to generate data on egg densities and parasitisation/predation rates.

Additional sampling will be conducted in sorghum fields to answer if grain growers can benefit from reduced pest levels in landscapes with a high percentage of Bt cotton in comparison with growers in landscapes with a high percentage of other crops. This question is highly relevant for grain growers and cannot be answered directly from the current project.

Data will be analyzed statistically in greater detail once both field seasons are completed and publications will be prepared thereafter. The second field season will improve the quality of the data dramatically and will allow us to publish the results in higher ranking journals.

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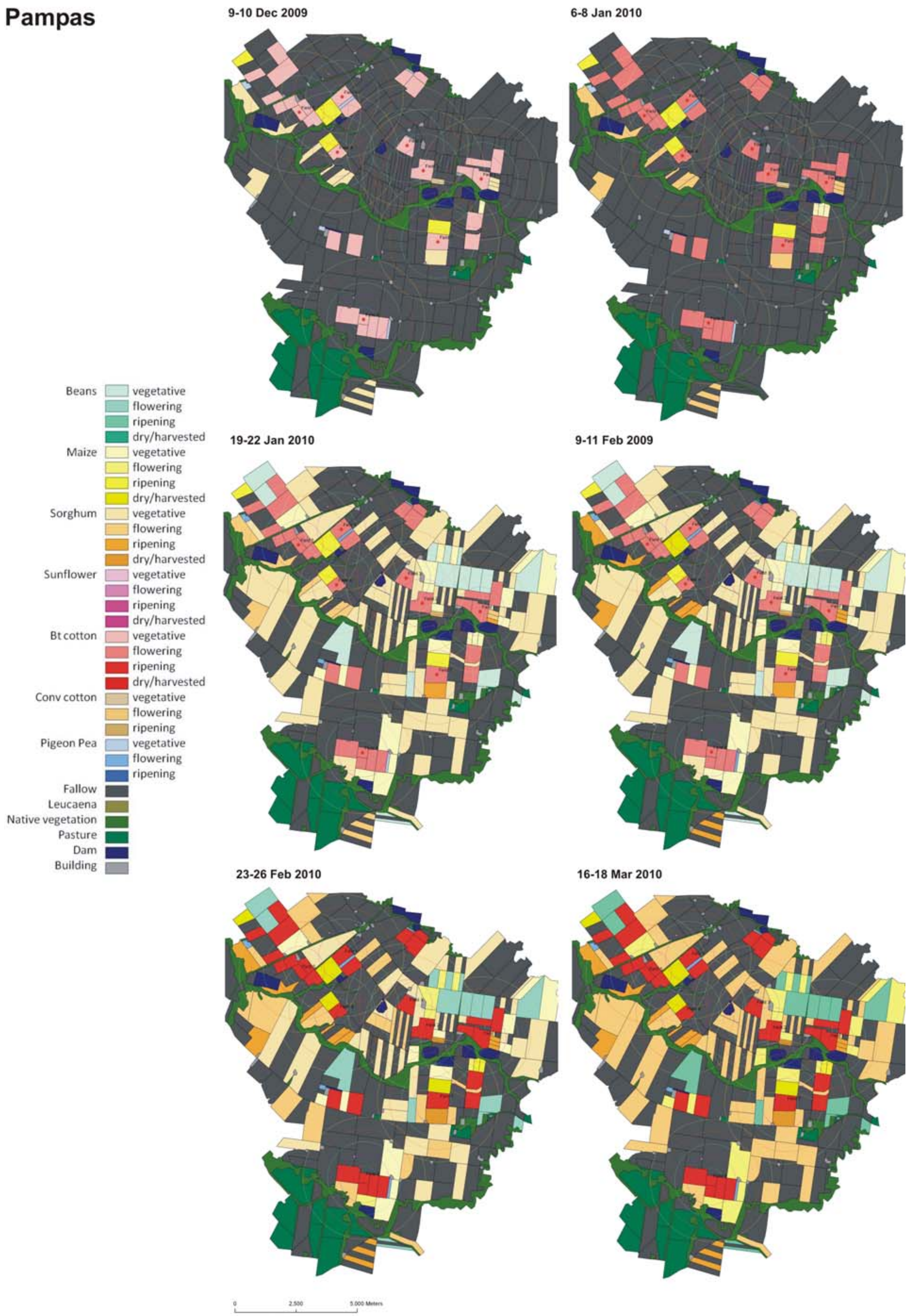
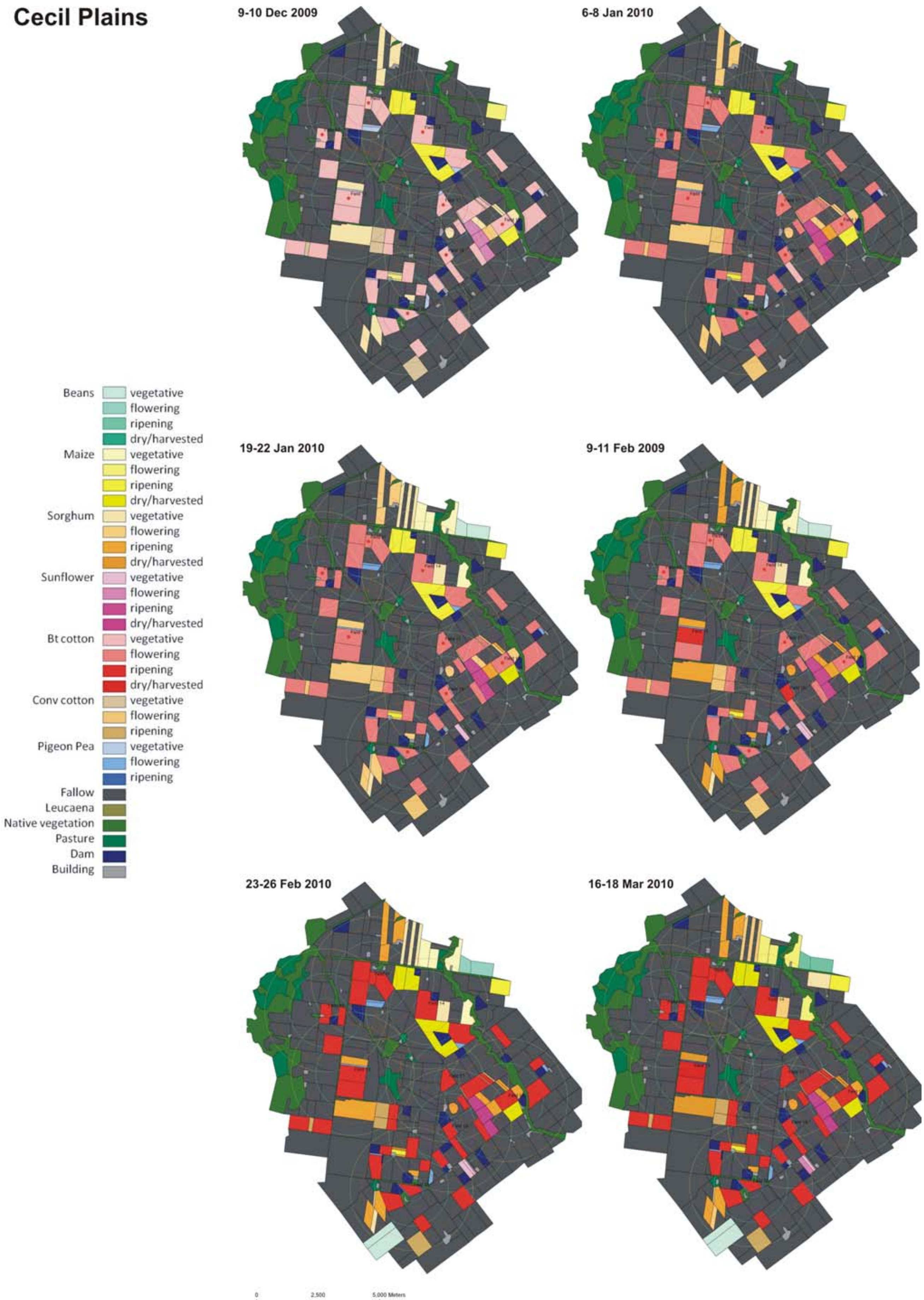
Annex 1

Crops and other landscape features during the sampling season 2009/2010. Fields selected for *Helicoverpa* spp. egg collections and egg cards are labelled. Landscape features around selected fields are calculated for circles with 0.5, 1.0, 1.5, 2.0, and 2.5 km radius.



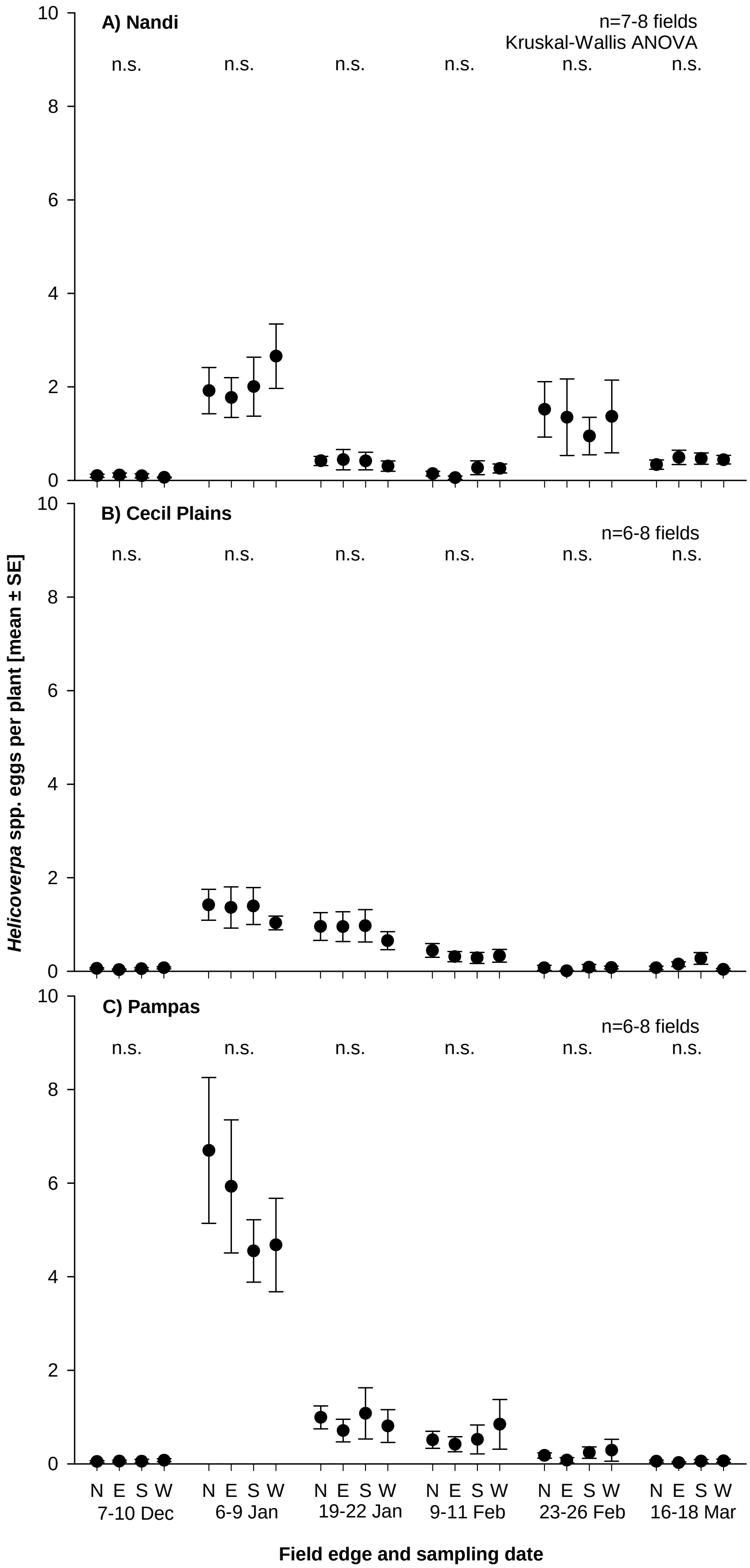

Cecil Plains
9-10 Dec 2009
6-8 Jan 2010
19-22 Jan 2010
9-11 Feb 2009
23-26 Feb 2010
16-18 Mar 2010
Beans
vegetative
flowering
ripening
dry/harvested
Maize
vegetative
flowering
ripening
dry/harvested
Sorghum
vegetative
flowering
ripening
dry/harvested
Sunflower
vegetative
flowering
ripening
dry/harvested
Bt cotton
vegetative
flowering
ripening
dry/harvested
Conv cotton
vegetative
flowering
ripening
Pigeon Pea
vegetative
flowering
ripening
Fallow
Leucaena
Native vegetation
Pasture
Dam
Building
0
2,500
5,000 Meters

Nandi


9-10 Dec 2009
6-8 Jan 2010
19-22 Jan 2010
9-11 Feb 2009
23-26 Feb 2010
16-18 Mar 2010
Beans
vegetative
flowering
ripening
dry/harvested
Maize
vegetative
flowering
ripening
dry/harvested
Sorghum
vegetative
flowering
ripening
dry/harvested
Sunflower
vegetative
flowering
ripening
dry/harvested
Bt cotton
vegetative
flowering
ripening
dry/harvested
Conv cotton
vegetative
flowering
ripening
Pigeon Pea
vegetative
flowering
ripening
Fallow
Leucaena
Native vegetation
Pasture
Dam
Building
0
2,500
5,000 Meters

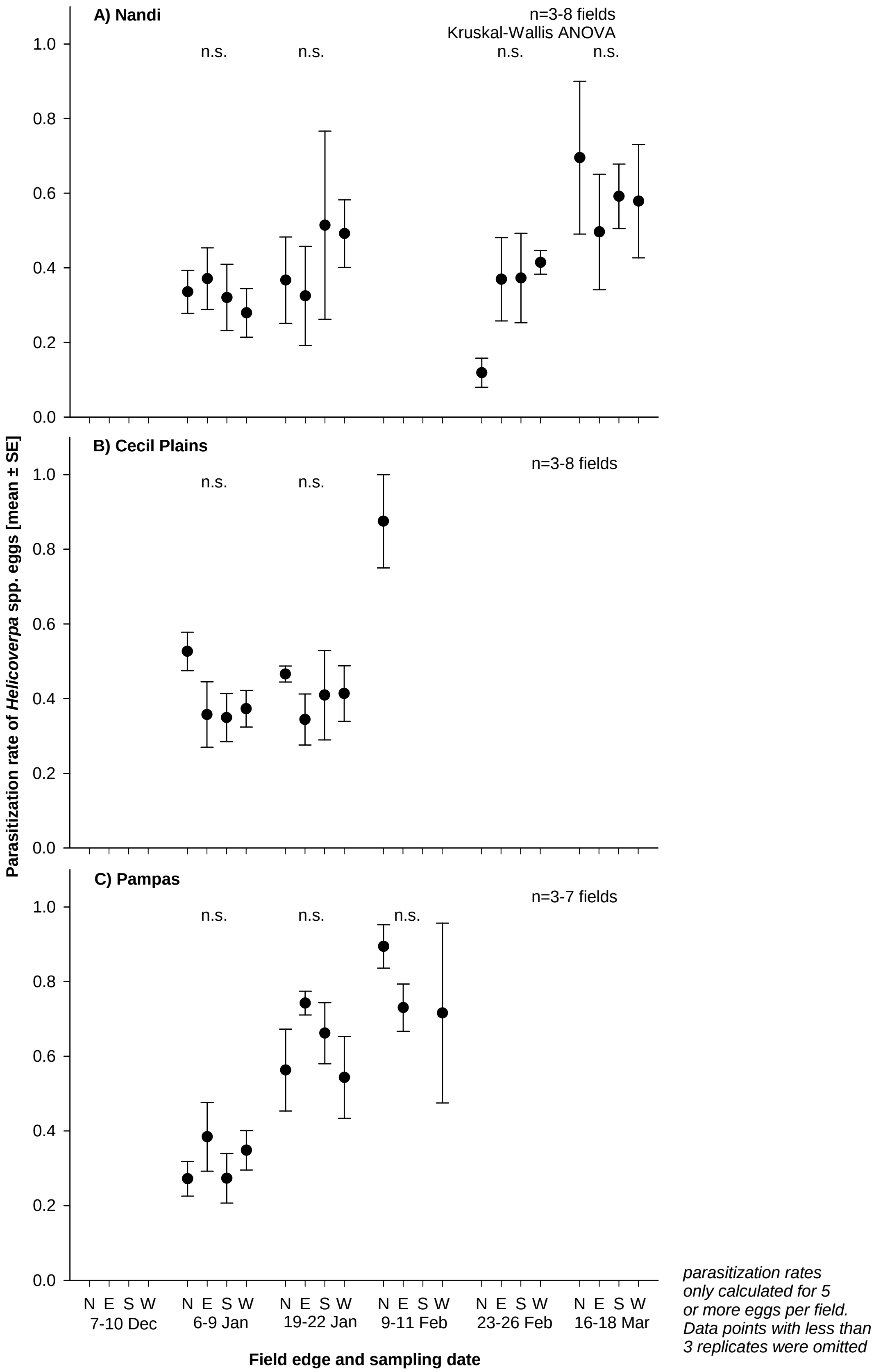
Annex 2A

Comparison of *Helicoverpa* spp. egg densities collected at different cardinal directions in cotton fields in the landscapes A) Nandi, B) Cecil Plains, and C) Pampas.



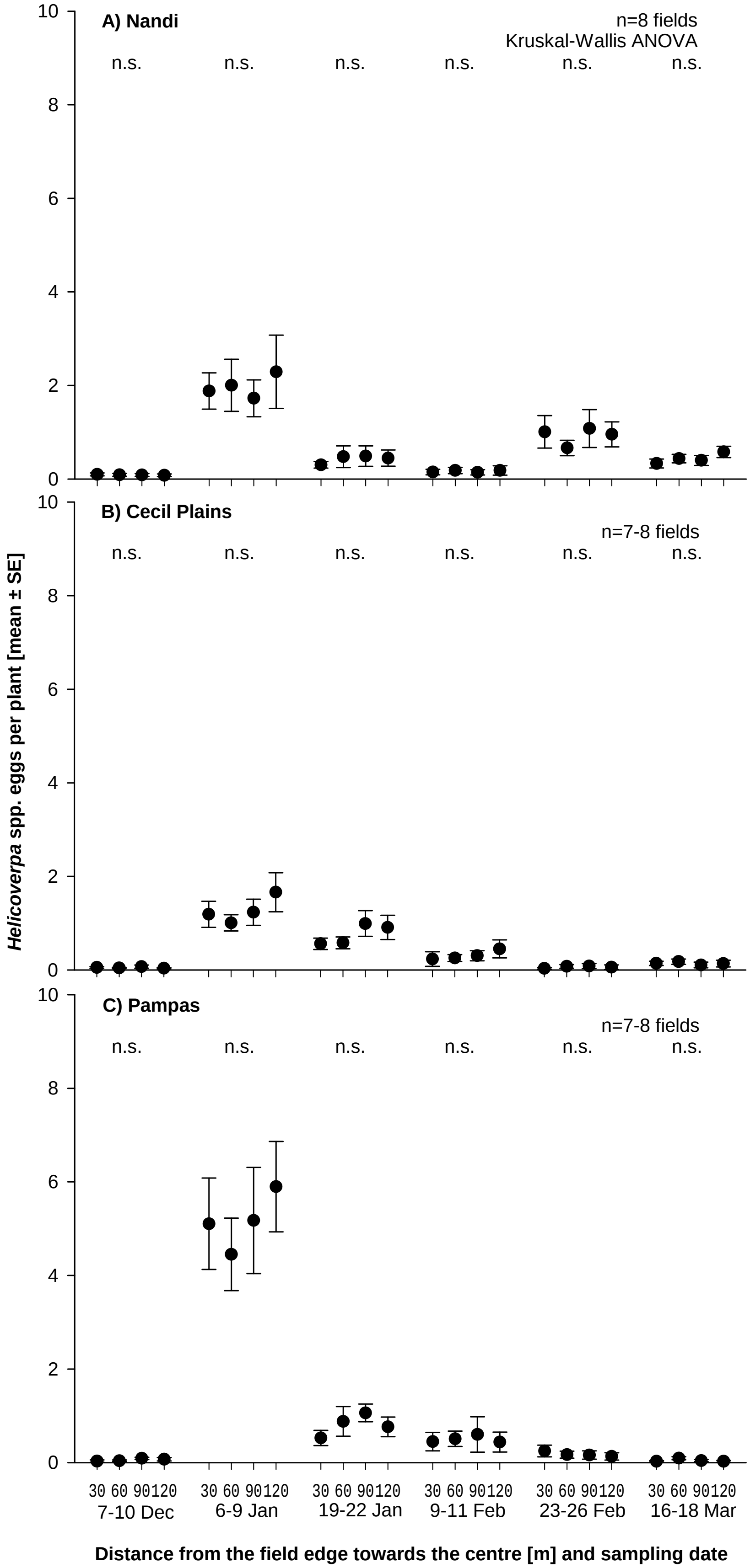
Annex 2B

Comparison of *Helicoverpa* spp. egg parasitization rates at different cardinal directions in cotton fields in the landscapes A) Nandi, B) Cecil Plains, and C) Pampas.



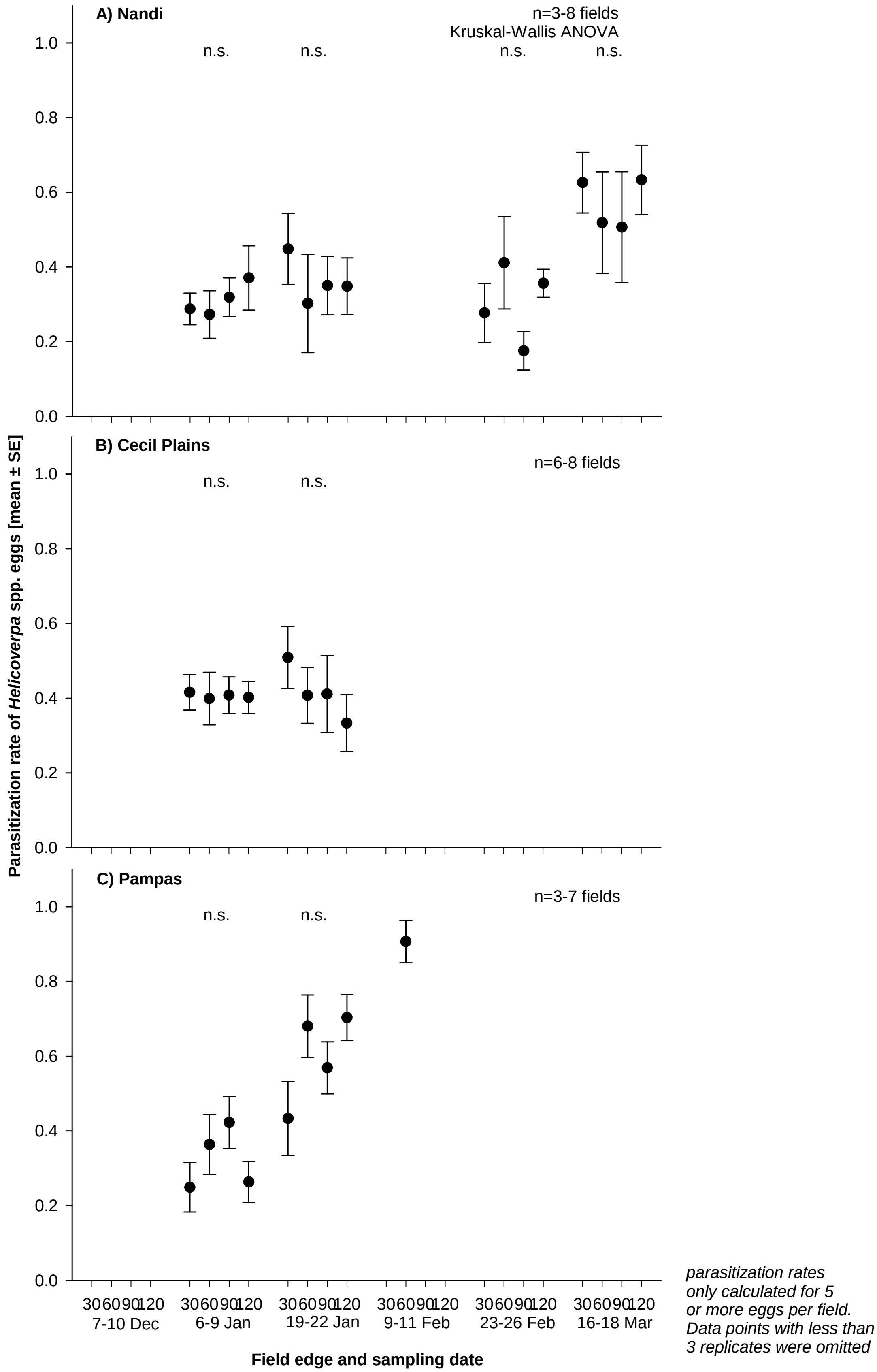
Annex 2C

Comparison of *Helicoverpa* spp. egg densities collected at different distances from the field edge in cotton fields in the landscapes A) Nandi, B) Cecil Plains, and C) Pampas.



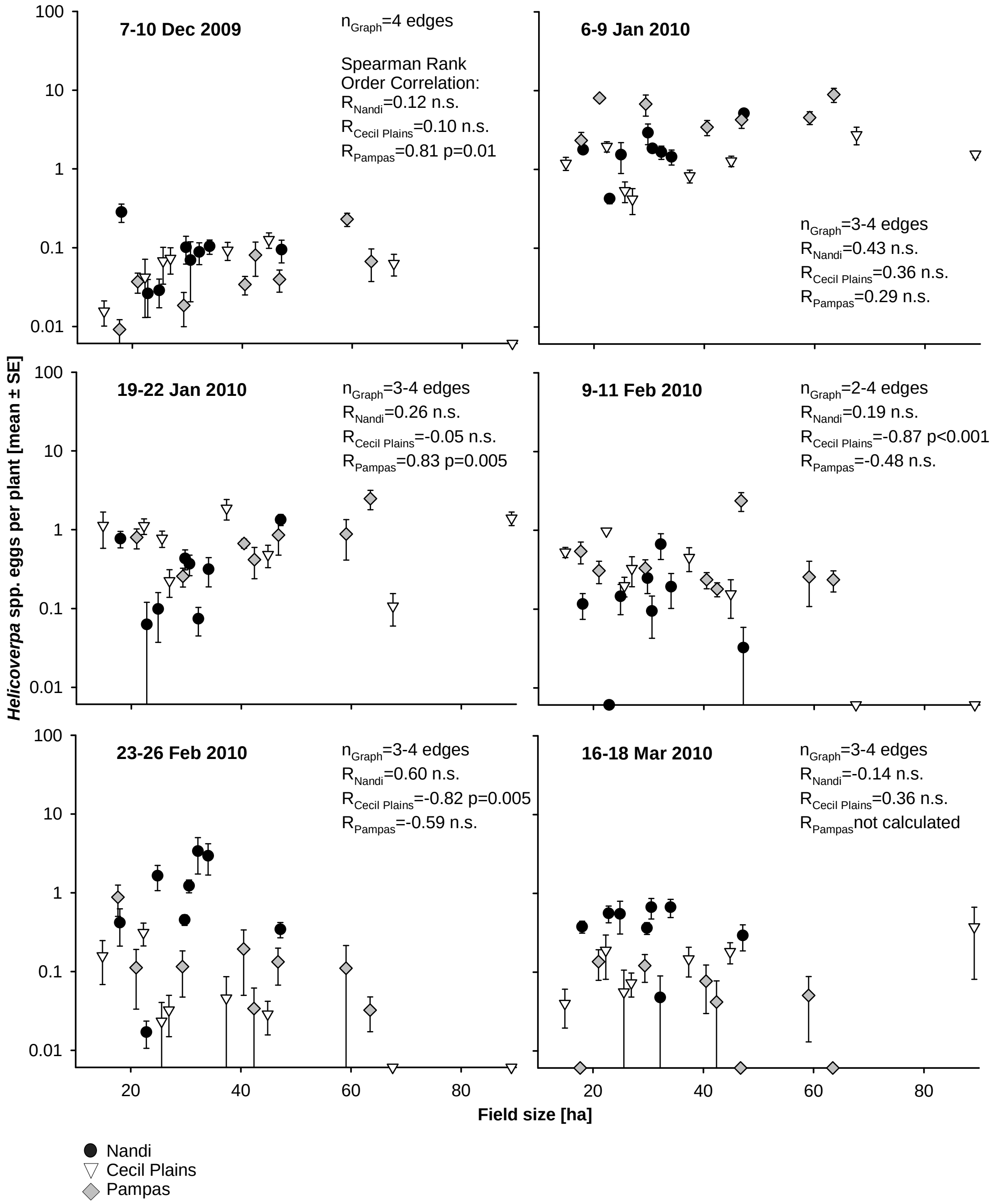
Annex 2D

Comparison of *Helicoverpa* spp. egg parasitization rates at different distances from the field edge of cotton fields in the landscapes A) Nandi, B) Cecil Plains, and C) Pampas.



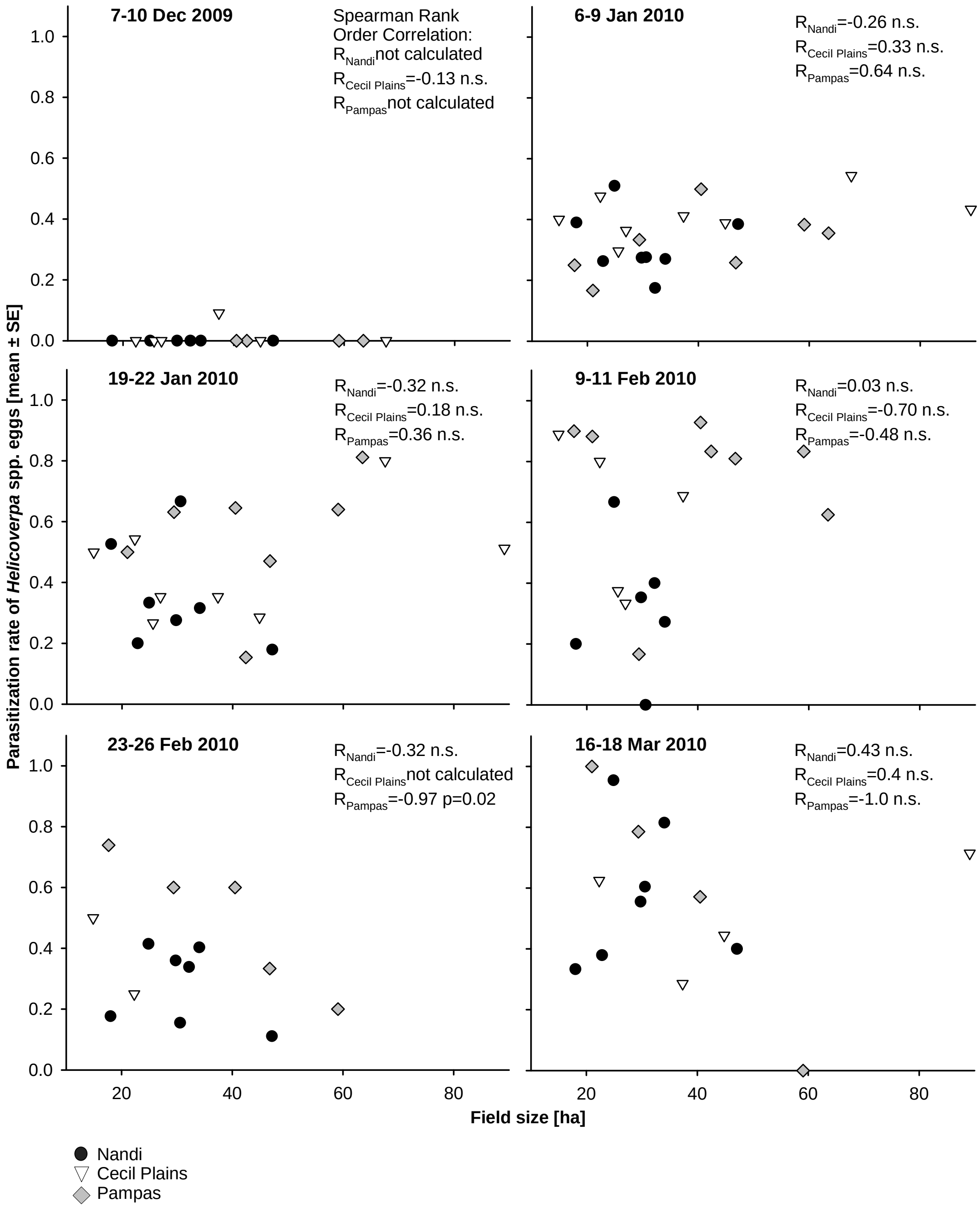
Annex 3A

Correlation of field size with *Helicoverpa* spp. egg density in the landscapes Nandi, Cecil Plains, and Pampas. For plotting 0 values on the logarithmic axis, 0.5 × lowest value (0.006098) was added to each data point.



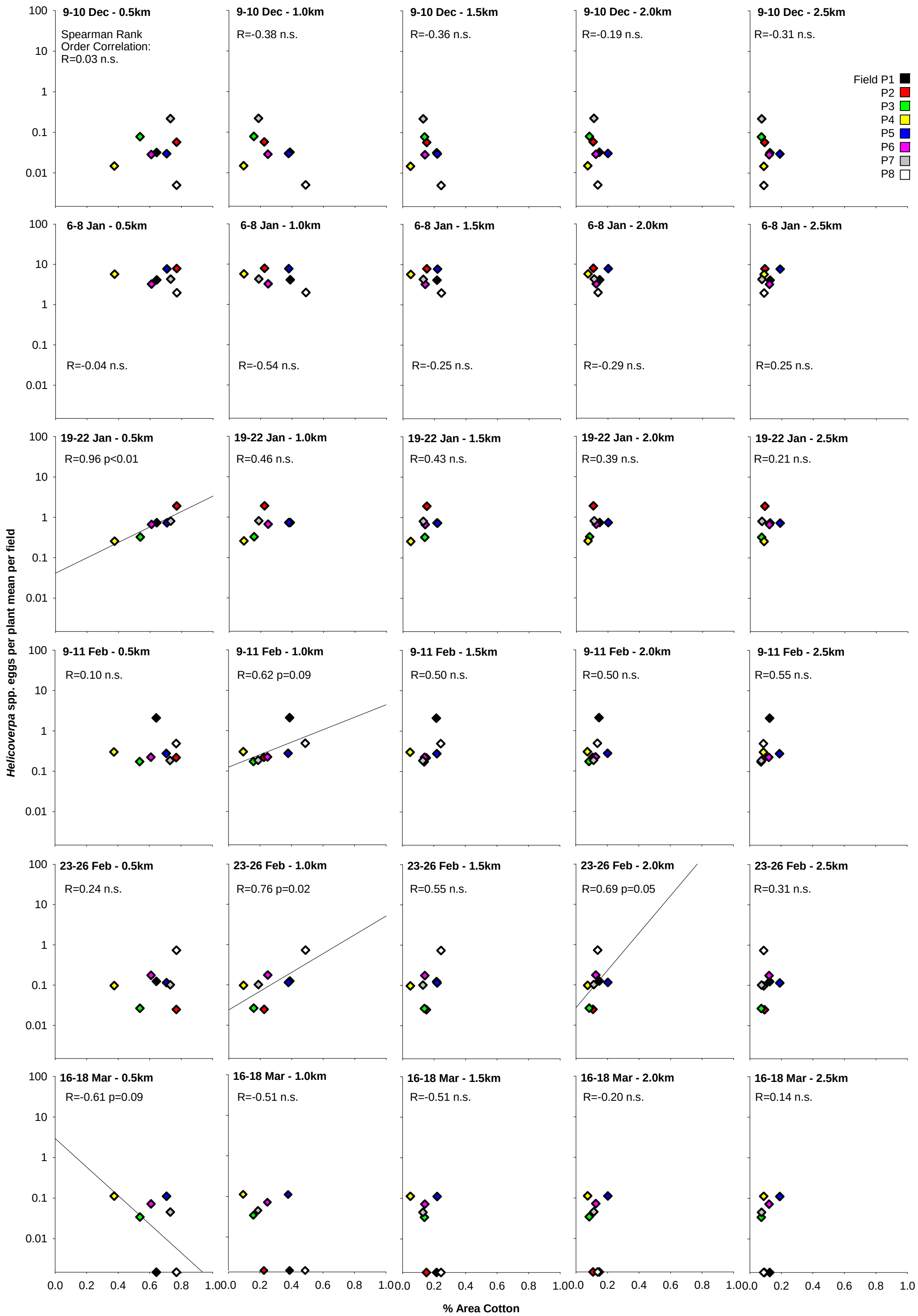
Annex 3B

Correlation of field size with *Helicoverpa* spp. egg parasitization rates in the landscapes Nandi, Cecil Plains, and Pampas. Correlations were not calculated for less than 4 data points.



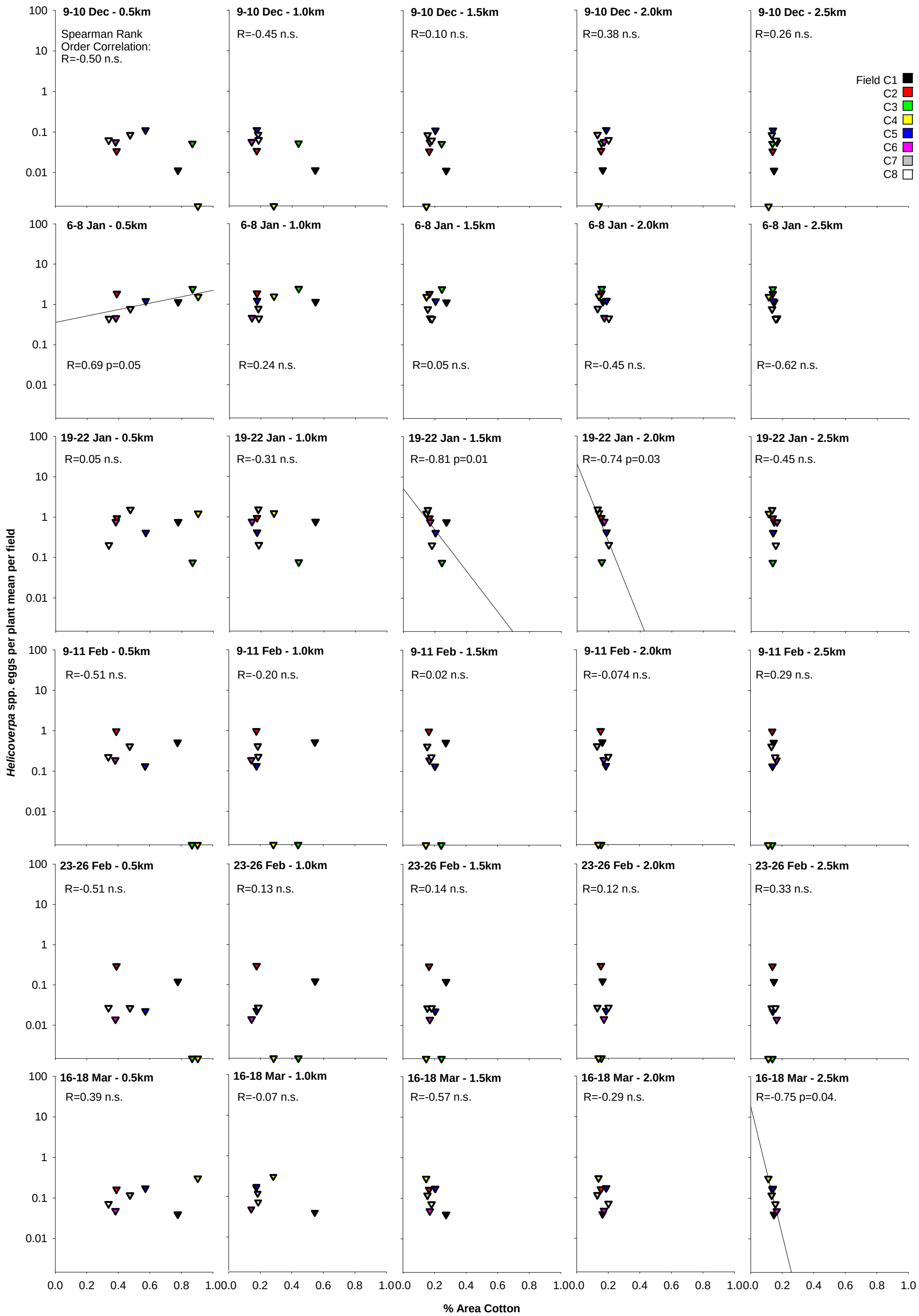
Annex 4A

Correlation of cotton area (in percent) within circles of different radia (0.5-2.5 km) around the focus fields with *Helicoverpa* spp. egg densities in those fields for the landscape Pampas.



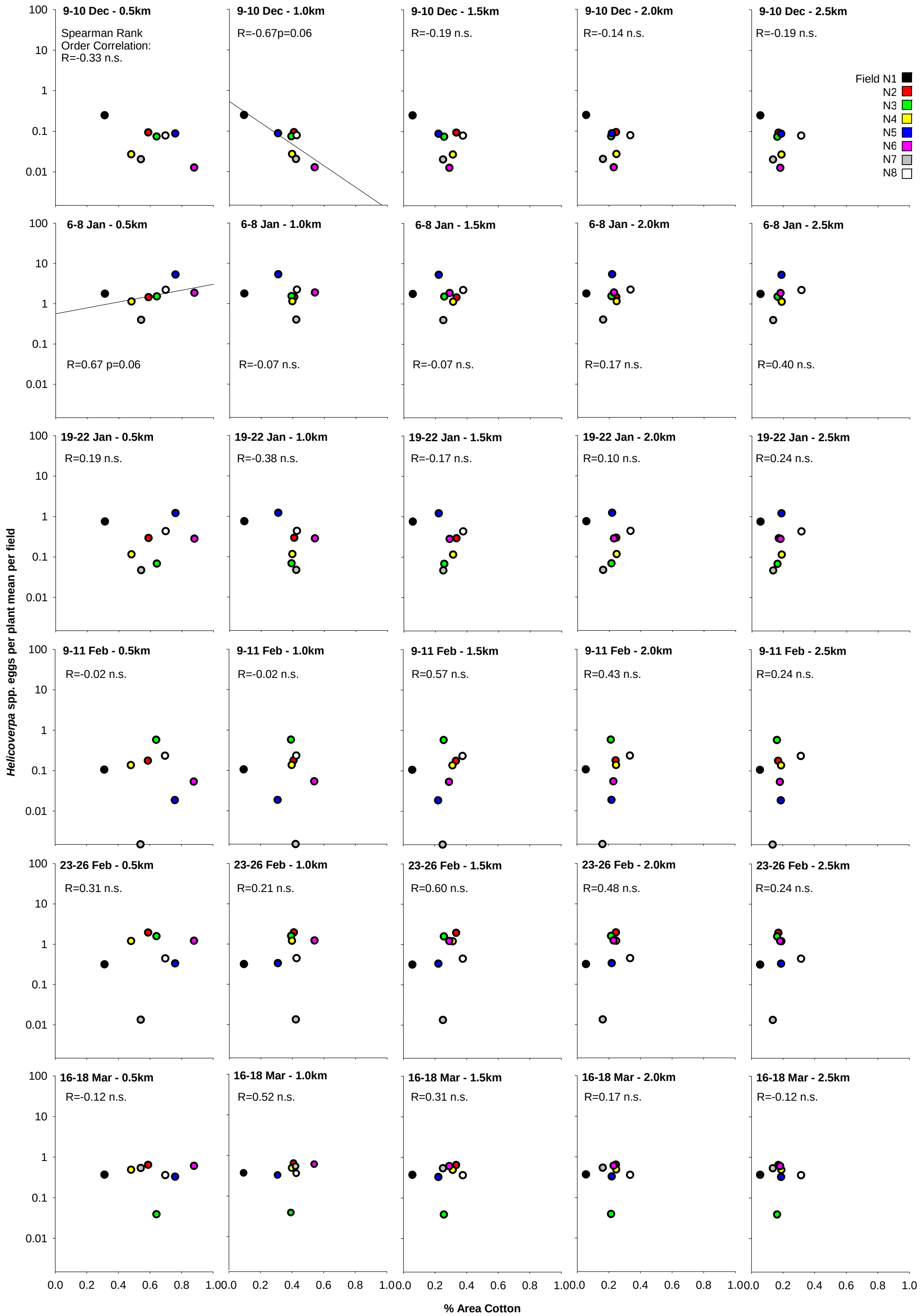
Annex 4B

Correlation of cotton area (in percent) within circles of different radia (0.5-2.5 km) around the focus fields with *Helicoverpa* spp. egg densities in those fields for the landscape Cecil Plains.



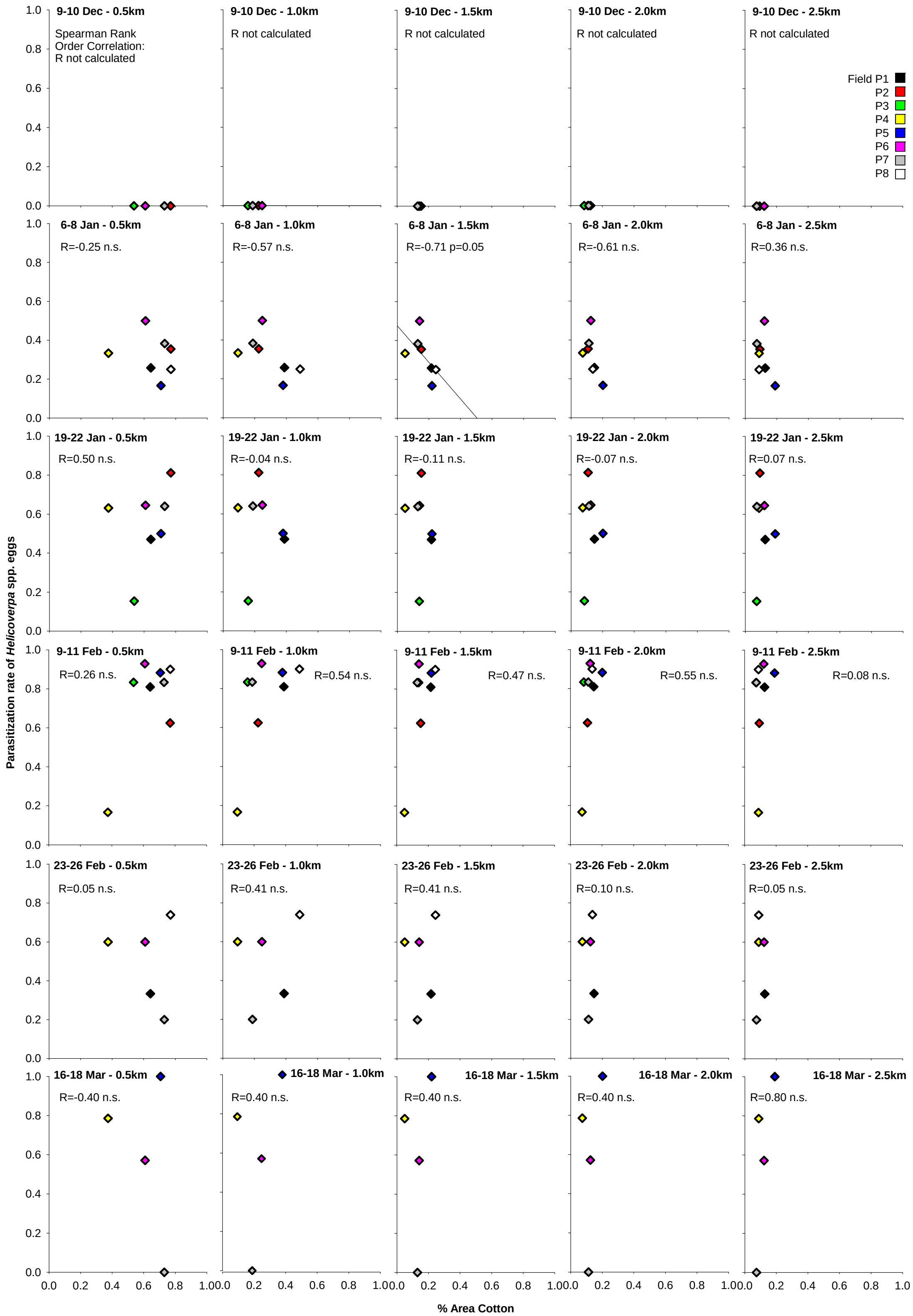
Annex 4C

Correlation of cotton area (in percent) within circles of different radia (0.5-2.5 km) around the focus fields with *Helicoverpa* spp. egg densities in those fields for the landscape Nandi.



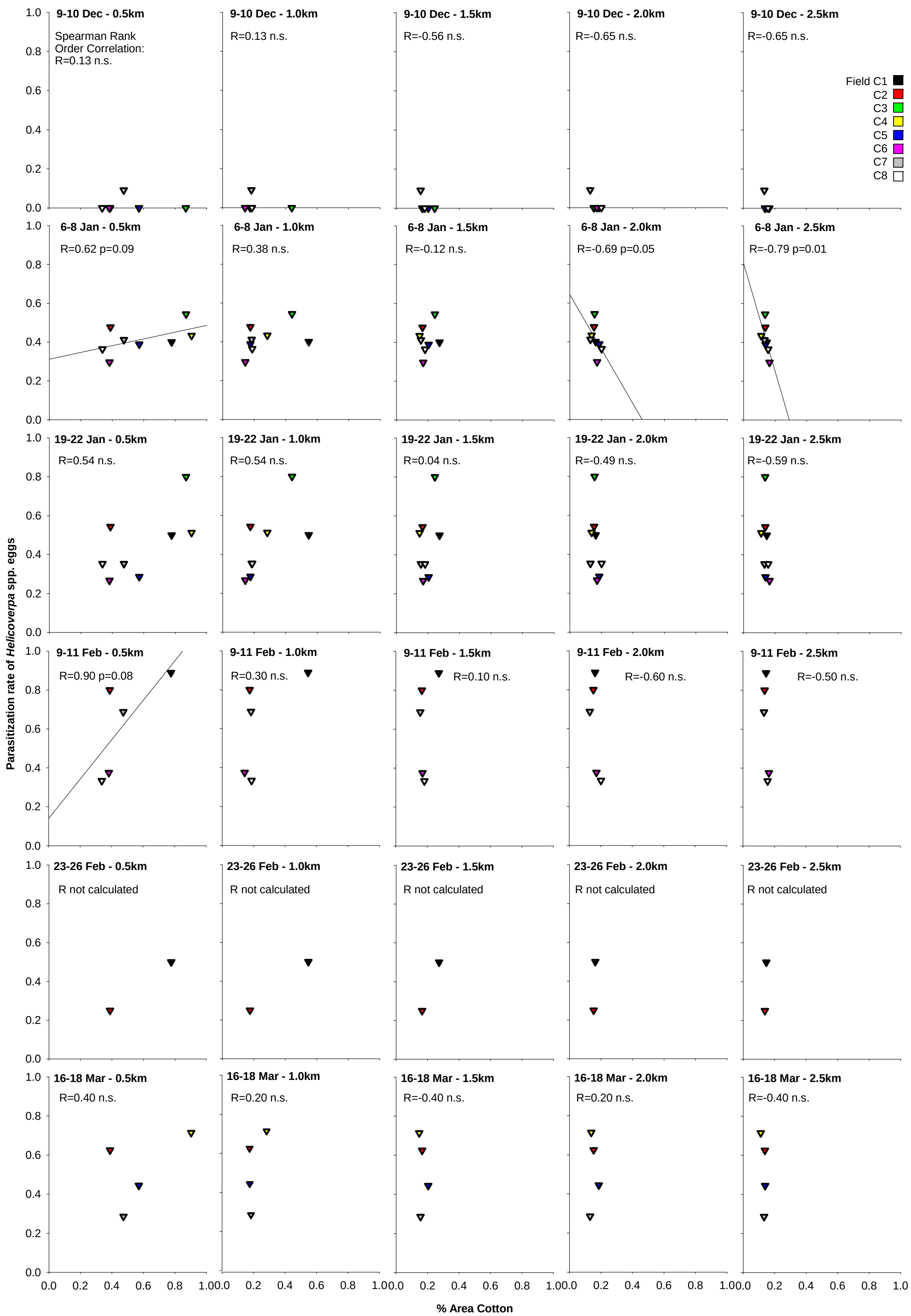
Annex 4D

Correlation of cotton area (in percent) within circles of different radia (0.5-2.5 km) around the focus fields with *Helicoverpa* spp. egg parasitisation rates in those fields for the landscape Pampas.



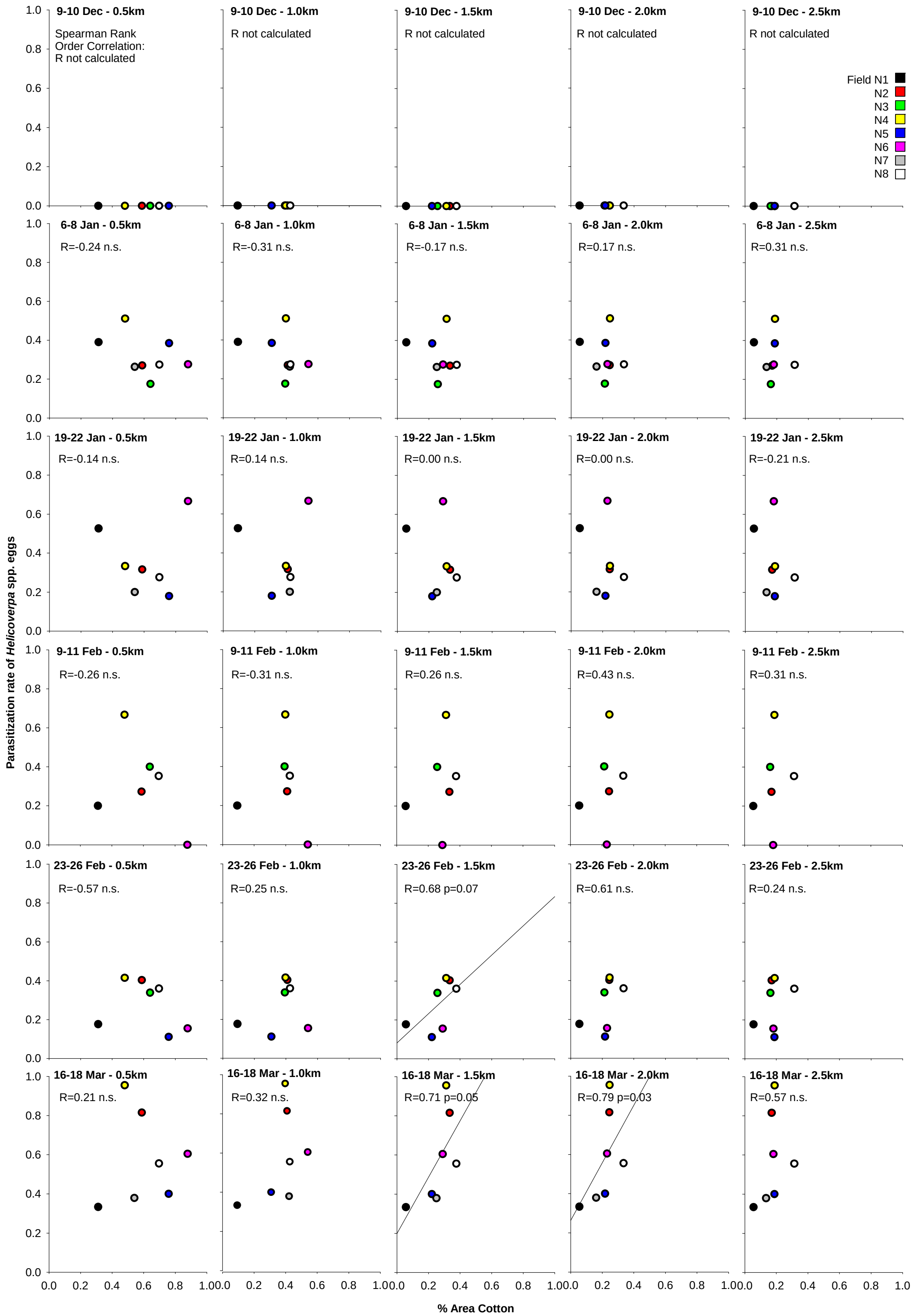
Annex 4E

Correlation of cotton area (in percent) within circles of different radia (0.5-2.5 km) around the focus fields with *Helicoverpa* spp. egg parasitisation rates in those fields for the landscape Cecil Plains.



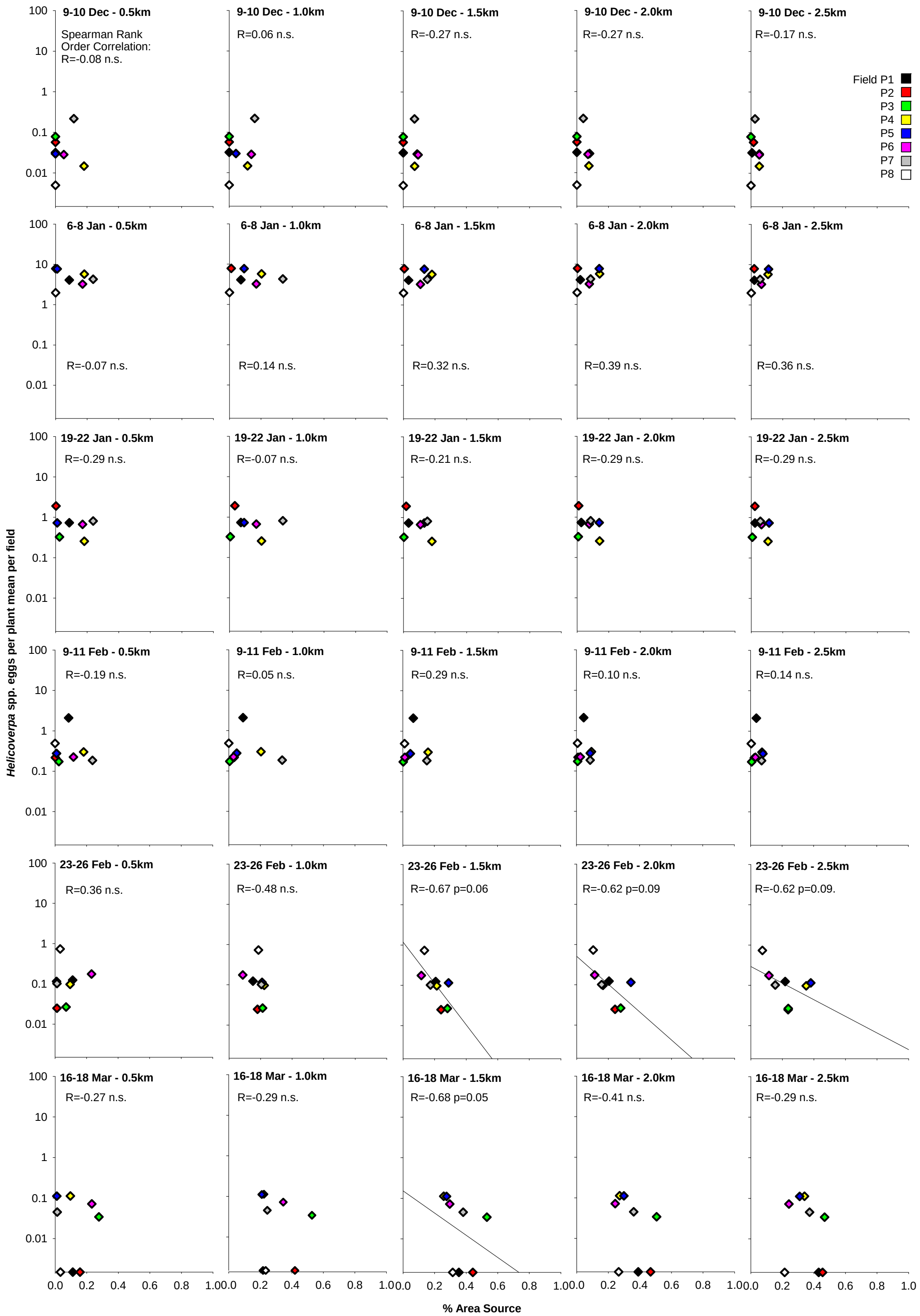
Annex 4F

Correlation of cotton area (in percent) within circles of different radia (0.5-2.5 km) around the focus fields with *Helicoverpa* spp. egg parasitisation rates in those fields for the landscape Nandi.



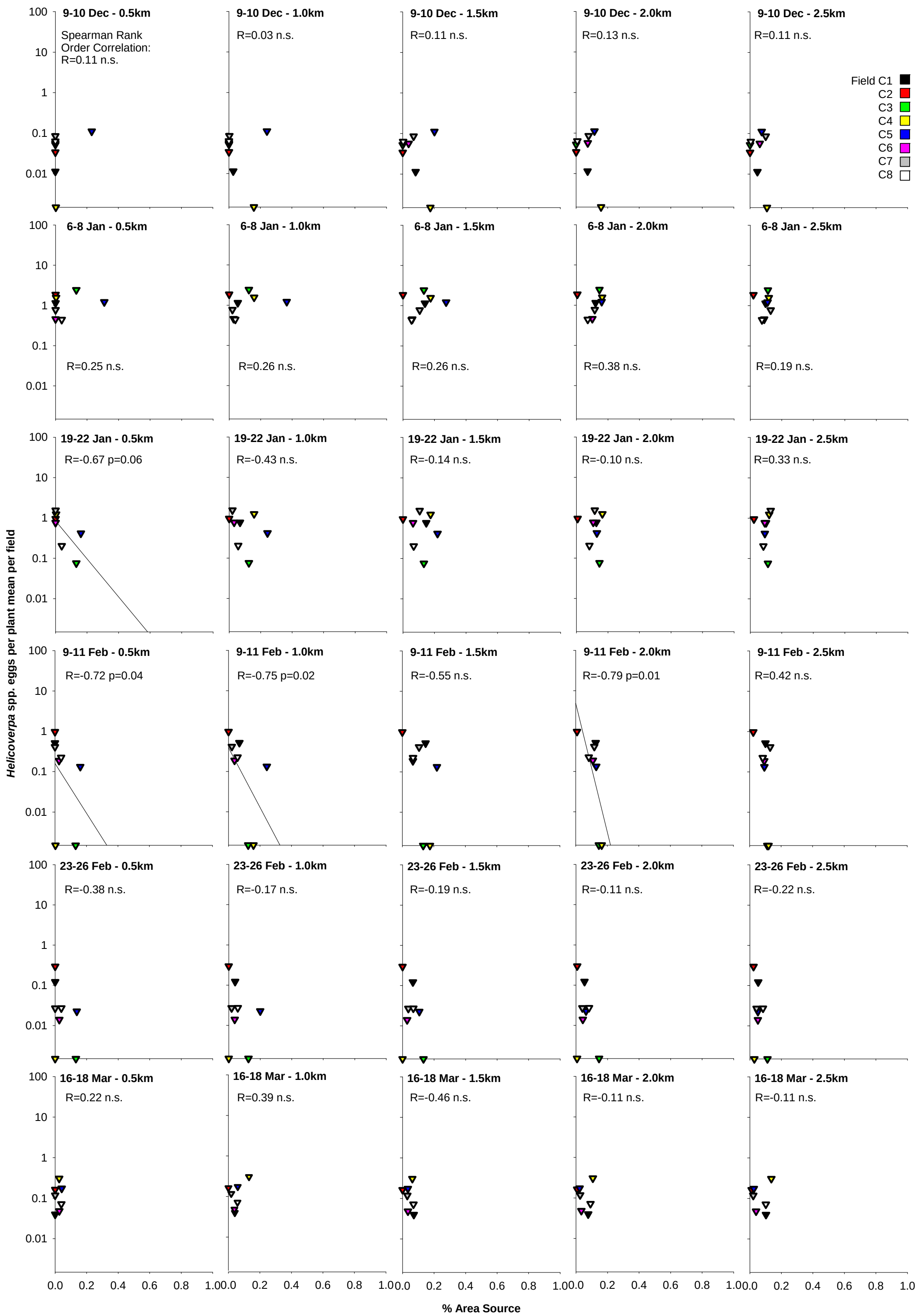
Annex 5A

Correlation of potential source crop area (in percent) within circles of different radia (0.5-2.5 km) around the focus fields with *Helicoverpa* spp. egg densities in those fields for the landscape Pampas.



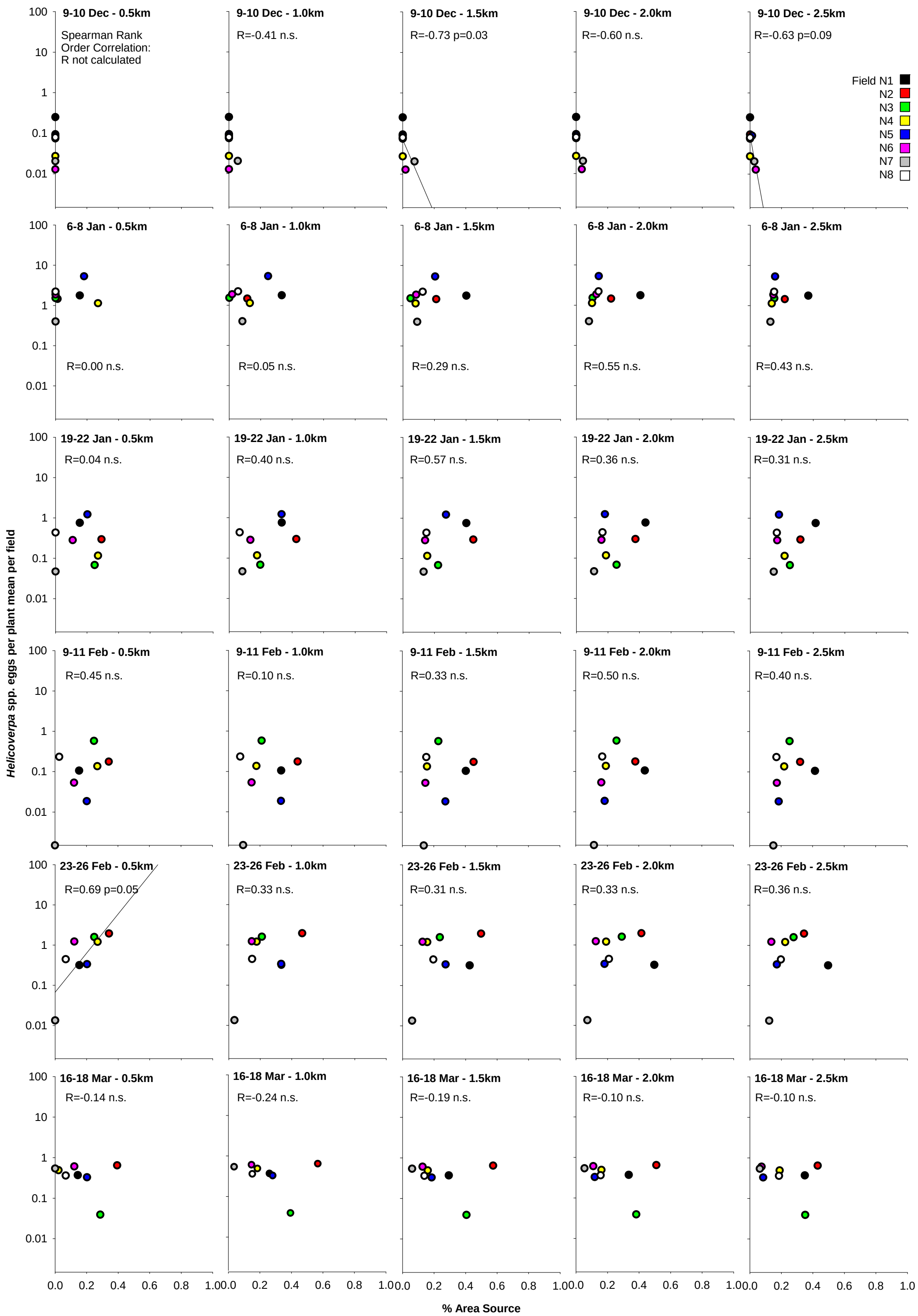
Annex 5B

Correlation of potential source crop area (in percent) within circles of different radia (0.5-2.5 km) around the focus fields with *Helicoverpa* spp. egg densities in those fields for the landscape Cecil Plains.



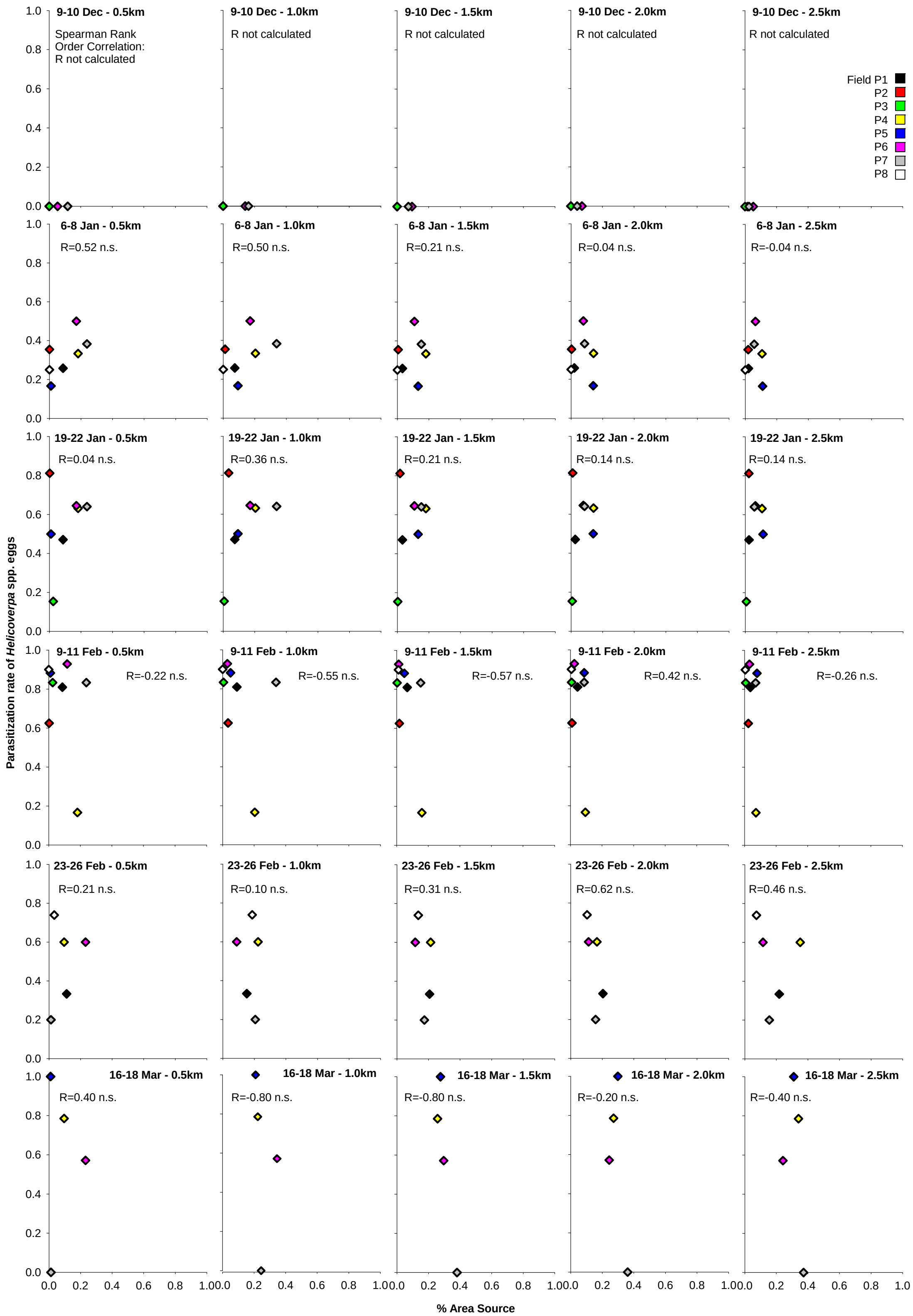
Annex 5C

Correlation of potential source crop area (in percent) within circles of different radia (0.5-2.5 km) around the focus fields with *Helicoverpa* spp. egg densities in those fields for the landscape Nandi.



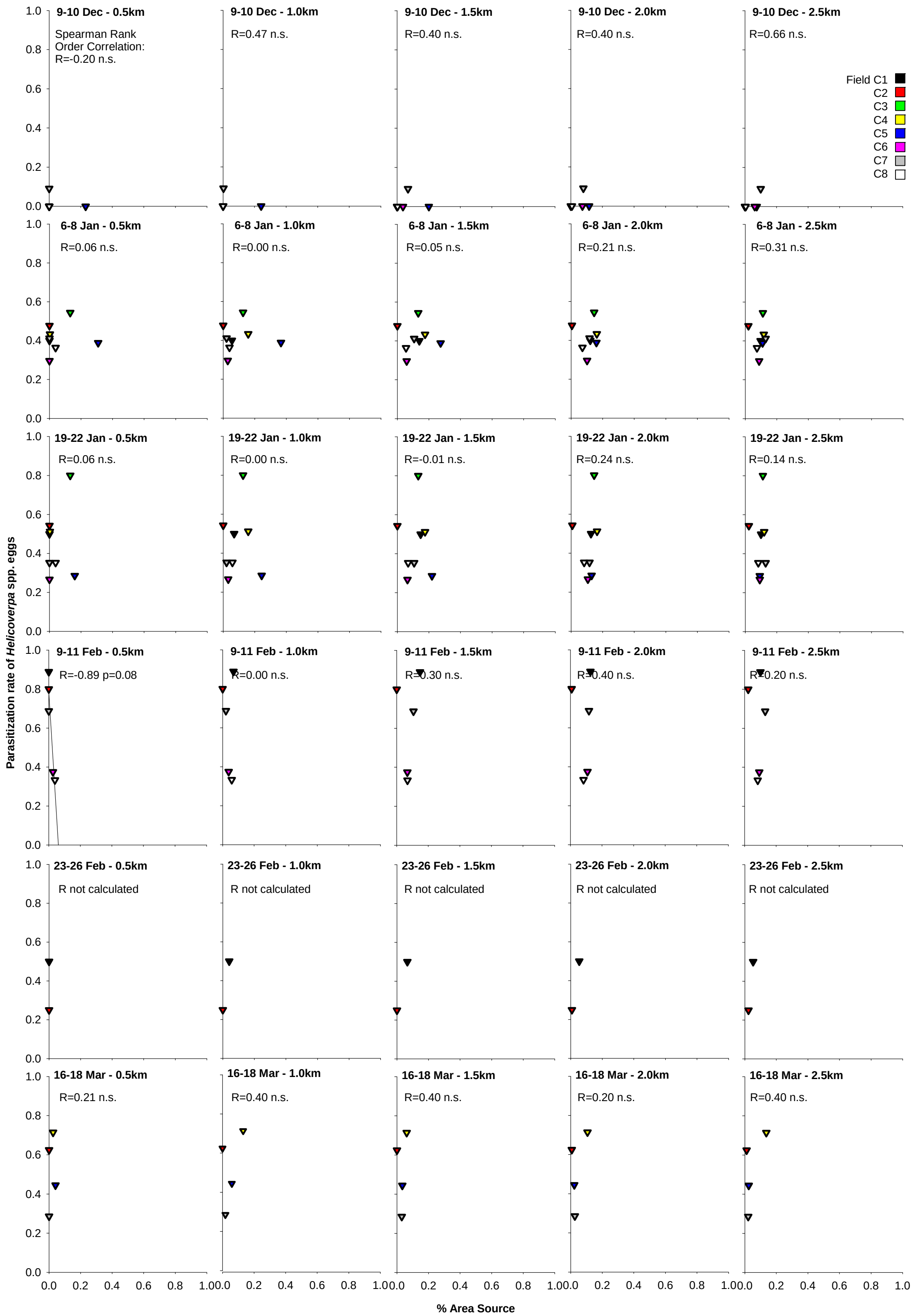
Annex 5D

Correlation of potential source crop area (in percent) within circles of different radia (0.5-2.5 km) around the focus fields with *Helicoverpa* spp. egg parasitization rates in those fields for the landscape Pampas.



Annex 5E

Correlation of potential source crop area (in percent) within circles of different radia (0.5-2.5 km) around the focus fields with *Helicoverpa* spp. egg parasitization rates in those fields for the landscape Cecil Plains.



Annex 5F

Correlation of potential source crop area (in percent) within circles of different radia (0.5-2.5 km) around the focus fields with *Helicoverpa* spp. egg parasitization rates in those fields for the landscape Nandi.